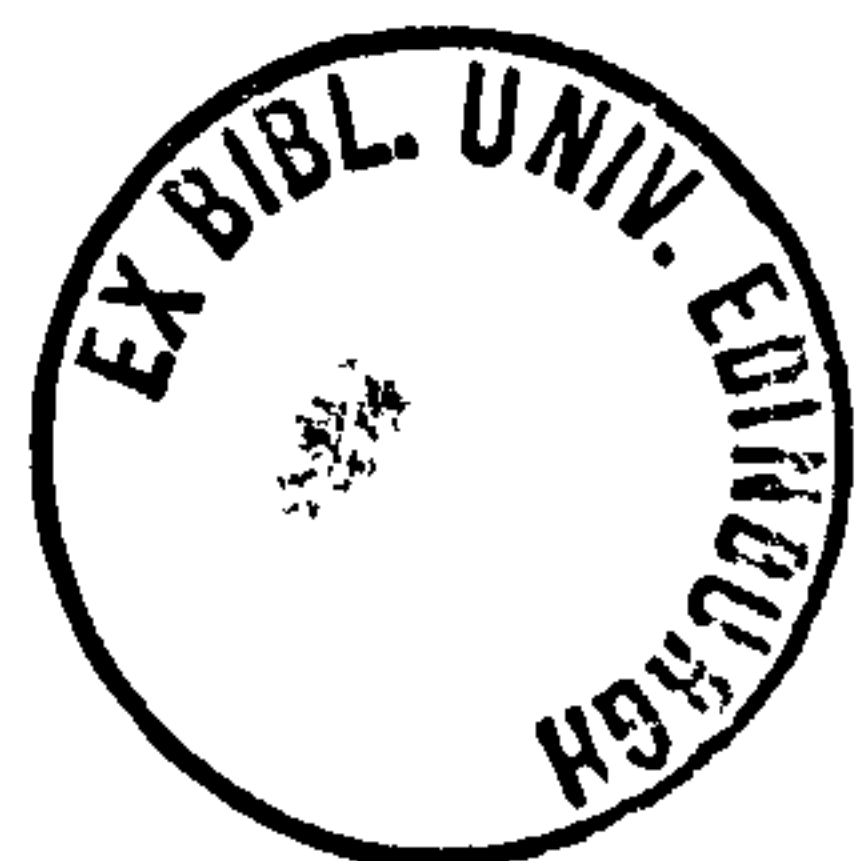


**An Investigation of Fluid Mechanical Influences  
on the  
Clotting of a Blood Analogue Fluid**

**by**

**John Randal Ernest Christy**

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Doctor of Philosophy  
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**With fond appreciation  
to  
my wife, Beverley  
and  
my parents, Randal and Cynorah Christy**

The work described in this thesis is the original work of the author, except where specific reference is made to other sources. It has not been submitted, in whole or in part, for any degree at any other university.

John Randal Ernest Christy

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## Preface

Whilst advances in both cardiac surgery and artificial heart valve design have greatly enhanced survival expectations following cardiac valve replacement, an ideal valve has yet to be found: Tissue valves are still liable to lipid deposition and calcification, and mechanical prostheses cause thrombosis unless accompanied by a sufficient level of anticoagulation therapy.

Thrombosis in flowing blood around mechanical prostheses can arise from both the materials of construction of the valve and the local hydrodynamics. Incidence of thrombus due to the former can be eliminated by the use of such materials as vitreous and pyrolytic carbon. From a haemodynamic perspective, stasis, stagnation, shear stress and mass transfer have all been proposed as correlates of clotting; but, as yet, no one has determined the relative importance of each.

The present method of assessing thrombogenicity of valves and similar devices is by implantation in animals. This, apart from being distasteful, is expensive, requires lengthy experimentation and leads to results that may not necessarily apply to the human vascular system. The use of blood *in vitro* is unsatisfactory for heart valve evaluation, principally because in order to avoid recirculation of clotted material a single-pass system using blood at a rate of about 30 gallons/hour is required. Also, methods of storage of blood may affect the blood chemistry causing blood *in vitro* to be at best an analogue for blood *in vivo*. In this thesis a blood analogue fluid for thrombogenicity assessment is investigated, both to determine its suitability as such and to identify the hydrodynamic conditions for deposition of clot from an enzyme-triggered coagulation.

Lewis proposed a milk mixture as being a suitable analogue fluid for the evaluation of thrombogenic potential of cardiovascular implants. In Hladovec's net experiment and Petschek's stagnation point flow chamber experiment the milk clot deposition patterns were found to be remarkably similar, both macroscopically and microscopically, to thrombus deposition from blood. When Bjork-Shiley and Starr-Edwards valves were tested with this milk mixture in an experimental heart pump, clot formed in the same regions of the valves as does thrombus when the valves are implanted in the human heart. Lewis, however, had difficulty controlling the temperature of the milk mixture and therefore could not, with certainty, differentiate quantitatively between the clot forming tendencies of the different valves.

To overcome this problem of temperature control, a new test rig was designed (and built commercially), capable of supplying the prepared milk

mixture at a constant temperature, in steady or pulsatile flow, to the object under test. An investigation of the deposition of coagulum on a variety of test objects, from both steady and pulsatile milk flows, has been conducted using this apparatus. The objects, solids of revolution of shapes used by Vorhauer *in vivo* were housed in a cylindrical test chamber to provide axisymmetrical flow. Clot found on the upstream face of the objects is thought to be adventitious being caused by vapour bubbles adhering to the object, by surface irregularities, or by impinging particles of clot dislodged from the test chamber wall further upstream. Deposited coagulum downstream is generally smoother and more uniformly spread around the object, indicating a more ordered deposition pattern. In steady flow, clot is found to deposit in the wake of the test-bodies, as in Vorhauer's experiments, but in pulsatile flow a rather strange clotting pattern is observed: An azimuthal band of clot adheres to the downstream side of both the tear-drop and the sphere whilst virtually no clot deposits downstream of the cone.

Residence time experiments in the same apparatus around the same test objects have revealed that the unexpected deposition in pulsatile flow occurs only in regions of stasis. Further analysis shows that whilst stasis is necessary for milk clot deposition, it is not a sufficient condition.

A modified Lee White Test, devised to determine the effects, if any, of agitation on the milk clotting reaction, indicates that whilst agitation has no apparent influence on the length of the induction period, its effect on the adhesivity of the final clot to a solid surface is profound when agitation occurs at the end of the induction phase. Thus stasis, and some aspect of agitation (most probably high mass transfer rates) in the vicinity of a surface are proposed as the concomitant conditions for clotting on that surface.



## **Chapter 1. Introduction**

### **1.1 The Need for Prosthetic Valves**

The human heart is essentially a double pump, the right-hand side, or right heart, pumping deoxygenated blood from the body's venous system out through the pulmonary system of the lungs, and the left heart pumping the oxygenated blood from the lungs out into the body's arterial system. Each half of the heart comprises an atrium, or filling chamber, and a muscular ventricle, or pumping chamber. Since fluid is expelled by muscular contraction, non-return or check valves are required at the inlet and outlet of the pumping chamber to cause the blood to flow throughout the body. In the right heart the valve between the atrium and the ventricle is known as the tricuspid valve and that at the exit of the ventricle is the pulmonary valve. The corresponding valves in the left heart are the mitral and aortic valves respectively. During systole both ventricles contract, the resulting increase in pressure causing the mitral and tricuspid valves to close and the aortic and pulmonary valves to open. Blood is thus expelled from the heart into the arteries. During diastole the ventricular muscle relaxes, the reduction in pressure causing the aortic and pulmonary valves to close, and blood which has been collecting in the atria during systole, flows into the ventricles.

Casci<sup>1</sup> and Braunwald<sup>2</sup> provide a definitive account of the diseases of the heart, with reference to the need for valve replacement. According to these authors, these non-return valves can fail in either of two ways; by stenosis or by incompetence. Stenosis is a narrowing of the valve orifice when open, usually due to fusion of adjacent cusps or calcification around the base of the cusps. Incompetence occurs when the valves stiffen or deform so as not to close fully on flow reversal, thus allowing a backflow of blood past the valve. Valvular stenosis arises usually as a result of congenital or Rheumatic heart disease leading to fusion of the commissures and cusps. Mitral stenosis rarely requires valve replacement, but valve replacement is usually recommended for adults with valvular aortic stenosis. Regurgitation can arise from congenital valvular deformation, deformation through diseases such as infective endocarditis, Rheumatic fever, trauma and syphilis, or calcification of the valve annulus through diseases such as systemic hypertension and diabetes. With regurgitation, replacement is preferable to repair of the damaged valve. Tissue valves are recommended for tricuspid valve replacement whenever such a measure is deemed essential.

The decision to operate on a diseased heart is generally made with reference to the New York Heart Association Classification<sup>3</sup>, surgery often being limited



to patients belonging to classes III and IV<sup>1</sup>, i.e. patients whose symptoms are apparent during ordinary activities of daily life. The success of prosthetic valve implantation is strongly influenced by the physical state of the patient at the time of operation and some surgeons have therefore recommended surgery for those in class II<sup>2,4</sup> (showing symptoms only after Prolonged exertion). Whilst some medical centres have followed this recommendation, a widespread acceptance does not appear to have been forthcoming, presumably because of the long-term risks of anticoagulation therapy<sup>5,6</sup> and the relatively short lifespan of current tissue valves<sup>7</sup>.

An ideal prosthetic heart valve must not only fulfil the function of the natural valve<sup>8</sup> but must also be compatible with both the vascular tissue and blood. The following criteria have been proposed as the main prerequisites of such a valve<sup>9,10</sup>:-

- i) The resistance to forward flow must be minimal to prevent the need for excessive pressures in the heart to maintain cardiac output. Therefore, the effective orifice size of the valve when open must be the maximum possible.
- ii) The valve must close rapidly at the onset of reverse flow to prevent regurgitation.
- iii) Seepage during diastole must be kept to a minimum.
- iv) The valve must conform to the structure of the heart. The size of the valve orifice and the area swept out by any moving parts must lie within limits imposed by the dimensions of the heart.
- v) The material of construction of the valve must be compatible with both the endothelium and the blood, being non-carcinogenic, non-antigenic and non-toxic.
- vi) The valve must be sterile.
- vii) The valve must be non-haemolytic.
- viii) the valve must be non-thrombogenic.
- ix) The valve must be durable (i.e. having low mechanical wear), preferably remaining operational for at least 25 years.

- x) The valve must not make excessive noise.

No valve, as yet, fulfills all these criteria. In the following discussion the main problems associated with each type of prosthetic valve are outlined.

## **1.2 Problems Associated with Current Prosthetic Valves**

A caged-ball valve was the first to be used successfully for human implantation, when it was inserted by Hufnagel, in 1952, in the descending aorta for the treatment of severe aortic regurgitation<sup>11,12</sup>. A similar valve was used by Starr and Edwards in 1960 to replace a diseased mitral valve<sup>13</sup>. Since then a number of valves of various designs have been proposed and tested with various degrees of success. However, no valve has, as yet, been discovered that remains free from complications for more than a decade<sup>7</sup>.

All prosthetic valves can be divided into two major classes; mechanical and tissue. Tissue valves in use at present are mainly Heterografts, usually of porcine or bovine tissue, with some homografts of Dura Mater<sup>11</sup>. Flexible synthetic materials, whilst being studied experimentally<sup>14,15</sup>, have not as yet made successful implants. These tissue valves are central flow valves, mimicing the cusp structure of the natural valves. Poppet, tilting-disc and hinged-disc valves are all used as mechanical prostheses, flow in the first type being peripheral, in the latter types semi-central.

The first tissue valves took the form of aortic homografts<sup>16</sup>. These did not require anticoagulation therapy, but rapid wear of the cusps and collagen dissolution led to incompetence<sup>11</sup>. The use of heterografts such as the Hancock and the Carpentier-Edwards Porcine Xenografts has been more promising, again requiring little or no anticoagulation therapy<sup>17</sup> (except in the mitral position where the risk of thrombosis is greater than in the aorta), though recent results suggest that the valves are prone to rapid deterioration in just under ten years<sup>18</sup>. Lipid deposition, fibrin deposition and calcification cause a stiffening of such valves<sup>19</sup> and degeneration of the tissue can lead to torn cusps<sup>20</sup>. The Ionescu-Shiley valve<sup>21</sup>, fabricated from bovine pericardium, now using a single sheet of tissue to minimise tearing resulting from stitching the cusps, and the Zerbini dura mater homograft are the only current tissue valve rivals of the porcine xenografts<sup>2</sup>. Anticoagulants are required for only 10% of patients receiving aortic heterografts, though they are necessary for approximately 50% of patients receiving mitral valves<sup>2</sup>. With or without anticoagulation therapy the reported incidence of thromboemboli with tissue valves is less than 2%/patient-year for aortic implants and 4%/patient-year for mitral valves<sup>22</sup>. Valve failure due to deteriorating or stiffening leaflets remains the main problem associated with these valves<sup>19</sup>.



Mechanical prostheses have also been prone to problems. Of the ball-valves presently in use (Starr-Edwards, Magovern-Cromie, Smeloff-Cutter)<sup>2,11</sup>, the most common is the Starr-Edwards valve. Early valves suffered from distortion and even splitting of the silicone rubber ball<sup>23</sup>, a fault that was initially rectified by the introduction of a hollow stellite ball. Between 1968 and 1980, one model of the Starr-Edwards valve<sup>24</sup>, the 4200 series, had struts coated in cloth to reduce the incidence of strut-related thromboembolism with metal runners on the inner face of the struts to prevent tearing of the cloth by the continual movement of the ball. This valve was withdrawn by the manufacturers in 1980 as it was found that it performed no better than the 1260 valve, having an improved silastic rubber ball and bare metal struts<sup>25,26</sup>. A major problem with these valves, apart from thrombosis, is the longitudinal projection of the struts into the downstream cavity<sup>2</sup>. This is particularly serious for patients with a small ventricle where protrusion of the struts into the intima can interfere with the oscillations of the ball<sup>27</sup>. Caged-disc valves were introduced as a solution to this spatial problem for mitral valve replacement. However, these proved to be highly thrombogenic, obstructive, haemolytic and prone to disc wear, and so are no longer used<sup>28,29,30</sup>.

Both types of poppet valve are obstructive to flow when placed in the aorta, leading to elevated pressure drops across the valve and high shear as the fluid is forced through the annulus formed by the poppet and the endothelial wall<sup>2</sup>. Tilting-disc valves, having a semi-central flow, are thus more suitable for aortic valve replacement. Of the tilting disc valves, (Bjork-Shiley, Lillehei-Kaster, Hall-Kaster) the Bjork-Shiley<sup>31,32</sup> is most commonly used<sup>33</sup>. Mechanical wear of the disc does not appear to be a problem, though strut weld fracture of the convexo-concave Bjork-Shiley valves<sup>34</sup> has led to the production of a monostrut valve, with the attachment ring and struts being formed in one piece by electrochemical machining<sup>35</sup>. Thromboembolism and thrombotic obstruction are still associated with such valves<sup>36</sup>, even during administration of anticoagulation therapy<sup>7,37</sup>.

The St. Jude Medical Prosthesis, a hinged leaflet, pyrolytic carbon valve, was hailed as being the first mechanical prosthesis not to require anticoagulation therapy<sup>38,39</sup>. However, implantation with antiplatelet drugs instead of anticoagulants has shown that the risk of thrombotic obstruction is high<sup>40,41</sup>. Evidence would suggest that this valve is likely to be similar to the Bjork-Shiley valve as far as thrombogenicity is concerned<sup>42,43</sup>.

Since failure of mechanical prostheses from structural causes is not a major occurrence in comparison with thrombotic incidents, future improvements in the design of such valves should focus on reduction of their thrombogenic propensity.

### 1.3 Thombosis - A Problem of Chemistry, Materials and Hydrodynamics.

Virchow, in 1856<sup>44</sup>, proposed a triad of factors important in thrombosis: Blood chemistry, surface chemistry and hydrodynamics. Investigation of the first of these has led to the use of anticoagulants, such as Warfarin and Coumarin, and antiplatelet drugs, such as Aspirin and Dipyridamole<sup>2</sup>. However, the suppression of thrombosis by means of chemical interference is not sufficient to totally inhibit thrombosis around prosthetic valves<sup>6</sup> and can, itself, be a cause of death<sup>22</sup>. Anticoagulation is not recommended for those, in particular, at risk of haemorrhage, such as pregnant women<sup>45</sup> and children<sup>46</sup>. An ideal valve should therefore require minimal use of drugs that suppress the normal clotting of blood.

Various research groups have investigated the compatibility of materials with blood<sup>47-51</sup>. *In vitro* tests will not be considered at this point as it is uncertain whether the chemical interaction of the blood with foreign materials *in vitro* is the same as that *in vivo*, especially if anticoagulants have been used during storage of the blood. A number of *in vivo* and *ex vivo* tests have been proposed; the canine vena caval Gott ring test<sup>52</sup>, insertion of flexible 'flags' or rigid 'swords'<sup>53</sup> into the canine right atrium, insertion of rings in the renal system<sup>54</sup>, suspension of a magnetic slug in the canine aorta<sup>55</sup> and impingement of blood, direct from the canine carotid artery, perpendicularly onto materials in Petschek's stagnation point flow chamber<sup>56</sup>.

Of the various *in vivo* compatibility tests devised, the Gott vena caval ring test<sup>52,57</sup> is the best documented for the widest range of materials. In this test, tubes constructed of, or coated with, the material under test are implanted in the canine vena cava. The quantity of thrombus adhering to the tube is determined in one test after two hours and in another, more stringent test, after two weeks. Results from these tests indicated that heparinised, anionic and 'inert' surfaces were all relatively athrombogenic, the inert surfaces being pyrolytic carbon, polyurethane and Stellite.

Doubts remain over the extent to which observations of material compatibility with canine blood apply to the human vascular system. Findings of Grabowski<sup>58</sup>, discussed in the next section, suggest that compatibility of materials with canine blood may bear no relation to that with human blood and, therefore, that the results of the Gott ring test should be treated with caution. It is generally accepted, however, that pyrolytic and vitreous carbon are among the most athrombogenic materials presently available for human implants<sup>59-61</sup>. The disc valves, currently used, have their moving parts coated in pyrolytic carbon<sup>37</sup>, and stellite is still used for the struts of the Bjork-Shiley and Starr-Edwards valves<sup>25</sup>.



In an effort to provide a more stringent test, Gott modified his original vena caval rings by the inclusion of, first, square-edged orifices and later orifices having a faired upstream face<sup>62,63</sup>. Materials that performed well in the original test with smooth rings were found to perform well in the square-edged orifice tubes so long as the diameter of the orifice was 6mm or more (in a 7mm diameter tube). Reduction to a diameter of 5.8mm resulted in thrombus formation, predominantly on the upstream face of the orifice. Fairing of the upstream face of the orifice with a 1mm radius of curvature rendered the test-piece athrombogenic once more. However with 5mm orifices thrombus formed both with the square-edged and the faired-edge orifice. Thus Gott demonstrated that thrombus formation is related to the nature of the flow near the surface and not just the surface's chemical properties.

Three further researchers in particular have studied the deposition of flow-related thrombosis *in vivo* or *in vitro*. Petschek<sup>56</sup>, Hladovec<sup>64</sup> and Vorhauer<sup>1,65</sup>. Petschek<sup>66,67</sup> in his stagnation point flow experiment observed that with a jet of arterial blood impinging on a flat surface of the material under test, at low flowrates, and therefore low shearing rates, a shear-limited white cell circle would deposit around the stagnation point and that in high shear wedges of thrombus formed around surface imperfections on an otherwise nonthrombogenic material. These thrombi would grow predominantly on their downstream side, resulting in a wedge formation with apex towards the stagnation point. Hladovec<sup>64</sup> devised an experiment in which a pulsatile flow of blood was circulated through a nylon mesh tethered across a tube. A thrombus formed on the downstream side of the net, not on the upstream or 'sieving' side, indicating that the clot was growing preferentially in this wake region, rather than by the accumulation on the screen of thrombi preformed in the bulk fluid. The rate of thrombus growth was found to be dependent on temperature but not on the mesh dimensions of the net. Vorhauer<sup>68</sup> inserted various shaped bodies of revolution into the canine descending aorta, measuring the weight of adherent thrombus after 45 minutes. Four of these objects, listed in order of increasing weight of thrombus, are the tear-drop, ball, disc and upstream-apex cone.

Many physical flow characteristics have been proposed as determinants of thrombus formation in such experiments, including shear stress, mass transfer coefficients, stagnation points and regions of stasis. Experimental evidence is beginning to allow discrimination among these possibilities. Recent work by Tiederman<sup>69</sup> using two-component laser Doppler Anemometry has shown that the maximum shear stress levels in the vicinity of a trileaflet valve are greater than those found near the Lillehei-Kaster disc valve prosthesis; this is apparently the reverse of the order of thrombogenicity for these valves, since it

is accepted that anticoagulation therapy must accompany implantation of the latter but not the former. Taylor<sup>70</sup> measured mass transfer coefficients in an apparatus identical to that used by Petschek and found that no correlation exists between the local mass transfer coefficients and the radius of the white cell circle reported by Petschek. Vorhauer's experiments, on the other hand, point to the wake region behind bluff bodies as being a favoured site for thrombus growth. Figliola<sup>71,72</sup> and Yoganathan<sup>73</sup> in their studies of flow parameters in the neighbourhood of prosthetic valves using Laser Doppler Anemometry have likewise linked regions of separated flow and stasis with observed sites of coagulation. Since this region is associated with high local mass transfer, stagnation points and stasis, identifying the effects of each flow property remains a problem.

#### **1.4 Present Thrombogenicity Testing of Prosthetic Valves.**

The currently accepted method of testing cardio-vascular devices, to predict their thrombogenicity in human patients, is to implant them in various species of animal, principally dogs and calves for prosthetic valves. Several considerations, apart from the wastefulness in terms of animal life, indicate that this is not the best technique:-

- i) High cost
- ii) Duration of experiment
- iii) Reliance of the valve's designer on the skill and consistency of the surgical team and support staff.
- iv) Inter-species variations in blood chemistry.

Grabowski<sup>58</sup> has shown that platelet adhesion from whole blood onto foreign surfaces forming the flow channel wall of an *in vitro* system, varies considerably from species to species (Fig. 1 ). The differences in platelet adhesion between species is biomaterial dependent, preventing even qualitative conclusions being drawn from the results. For instance, more human platelets adhere to compressed Gore-Tex than to Avcothane 51, whereas with canine platelets the reverse is true.



PLATELETS/mm <sup>2</sup>				
(Mean ± SE,N)* After 10 mins of Blood Flow at a Surface Shear Rate of 986 sec <sup>-1</sup>				
Species	Cuprophane PT-150	Avcothane 51	Compressed Gore-Tex	Fluorinated Ethylcellulose
Human	< 100 (4)	< 100	5,600 ± 1,500(4)	5,600 ± 5,000(4)
Dog	27,400 ± 4,600(5)	19,400 ± 9,300(4)	3,600 ± 1,600(4)	5,500 ± 3,500(4)
Rabbit	78,400 ± 6,400(4)	-	-	-
Calf	< 100 (4)	-	13,500 ± 9,800(5)	-
Baboon, Macaque, Hog, or Sheep	< 100 (4) <sup>†</sup>	-	-	-

\*N is the number of different subjects or animals.  
<sup>†</sup>N = 4 for each of the 4 species listed.

**Fig. 1** Table showing levels of platelet adhesion in different species<sup>58</sup>

v) Intra-species variations in blood chemistry.

Gott<sup>53</sup>, in inserting 'flags' and 'swords' of various materials into the canine right atrium, found difficulty in obtaining reproducible quantities of clot deposition from one dog to the next, when duplicating tests with each material, even though the same surgical procedures were meticulously followed throughout. For this reason, Gott chose the vena caval ring test as his standard compatibility test, since it yielded more consistent results. Uncertainty remains, therefore, over the reproducibility of thrombogenicity tests involving the implantation of prosthetic valves. Thus a statistically significant sample of animal tests is necessary before conclusions are drawn from such implantations.

vi) Inter-species variations of a hydrodynamic nature.

The mean systolic flowrate varies between species, as does the size of the heart. The effect of the differences in local flow properties, arising from such variations, on thrombosis around the valves is unknown. In the use of calves the heart will still be growing as the calf matures, leading to a changing environment during the course of the test. In dogs the heart rate is about twice that in man<sup>74</sup>. Coupled with the inertia of the valve, this will almost certainly lead to a change in the functional performance of the prosthesis.

Thus, if comparative testing of prostheses of various design is to be achieved, an alternative procedure, affording greater consistency, is desirable.

### 1.5 Milk as a possible Blood Analogue Fluid.

Since flow properties conducive to thrombosis are difficult to isolate and animal experimentation is undesirable, an analogue fluid for the experimental determination of thrombogenic tendency of implant devices has been proposed to facilitate the making of *in vitro* observations. The clotting of blood is complex, a cascade of reactions having been postulated to describe the mechanism leading to the conversion of prothrombin to thrombin<sup>75-77</sup>. Thus the probability of finding a blood analogue capable of mimicing the complete clotting sequence is remote. However, if it is assumed that *in vivo* the presence of foreign material in the sewing ring and the valve, and of damaged intima at the sewing ring, is sufficient to initiate the cascade of reactions culminating in the conversion of prothrombin to thrombin, then the blood flowing across the valve is therefore in a hypercoagulable state, and only the latter stage of the clotting reaction, that of the action of thrombin on fibrinogen, need be considered. This hypercoagulable state of the blood is plausible in that not all the blood is expelled from the ventricle during systole, so that some blood having made contact with the foreign surface of the valve will mix with the fresh blood entering from the atrium. Since thrombin enhances the action of several of the blood factors leading to the conversion of prothrombin to thrombin<sup>75</sup> this reaction is in some senses autocatalytic and the concentration of thrombin will increase before the next ejection. This hypercoagulable blood on passing through the valve appears to clot only at specific sites, such, for example, as the inside of the sewing ring and the downstream face of the struts of the Starr-Edwards Prosthesis<sup>78</sup>. It is believed that if these sites with flow patterns favourable to deposition can be eliminated, by suitable valve design, then thrombosis will not occur.

The blood analogue fluid chosen is therefore intended, not to mimic the cascade of reactions leading to the formation of thrombin, but only the action of thrombin on fibrinogen and the subsequent formation of a fibrin thrombus. Heated milk to which rennet (containing the enzyme Chymosin) and Calcium Chloride are added is one possible analogue<sup>79,80</sup>. Chemically the final blood and milk clotting reactions are similar, both involving the enzymic cleavage of a soluble protein to yield an insoluble protein and soluble glycopeptides<sup>81,82</sup>, the insoluble protein undergoing a second order polymerisation reaction to form a fibrous mesh. On a macroscopic scale both reactions exhibit an induction period during which no solid material is visible followed by a rapid coagulation to form a structured clot. Lewis has shown that the behaviour of this analogue in the stagnation point flow and Hladovec's net experiment<sup>81</sup> is remarkably similar to that of blood and that the milk curd formed around the Bjork-Shiley



and Starr-Edwards prostheses in an experimental heart chamber occur at the same sites as are prone to thrombosis *in vivo*<sup>82</sup>.

In this thesis, the present knowledge of flow-related thrombosis is reviewed, the design and commissioning of equipment for the preparation of the activated milk mixture is described, and, by means of the blood analogue, an investigation of the fluid dynamical correlates of clotting is presented.

## **Chapter 2. Thrombosis, Haemostasis and Haemolysis**

### **2.1 Haemostasis**

Haemostasis is the normal process of blood clotting by which the body prevents bleeding from wounds of significant size. A complex series of chemical reactions is initiated by damage of the vascular endothelium, resulting in the formation of a fibrin mesh in which blood platelets, erythrocytes and leucocytes are trapped<sup>75,77</sup>.

Following injury, the first observation is that platelets adhere to the endothelial connective tissue, including the basement membrane and collagen fibres<sup>76,83</sup>. This results in a platelet plug and the release of chemicals from the platelets. If the lesion is minor and the vessel wall small, this plug may be sufficient to halt the blood loss from the vessel and the clotting process proceeds no further. When the damage is greater, or the vessel is large, the second observation is that serotonin, released from the vessel wall and the aggregated platelets, causes constriction of the injured vessel. Thirdly, the coagulation process is triggered, involving both intrinsic and extrinsic systems. Collagen, elastin and other cellular components such as tissue factor are linked with this triggering process<sup>84</sup>. The resulting fibrin forms a mesh around the aggregated platelets giving strength and rigidity to the clotted plug. Finally, the fibrinolytic system is activated causing degradation of the fibrin clot as healing and regeneration of the vessel wall occur<sup>85</sup>.

In 1964, following many physiological and clinical studies, MacFarlane<sup>86</sup> and Davie and Ratnoff<sup>87</sup> postulated the cascade and waterfall theories of coagulation respectively, in which clotting is described as a series of proenzyme-enzyme conversions, ending in the production of thrombin, the catalyst for the fibrinogen-fibrin reaction<sup>88,89</sup>. Modifications have subsequently been made to these theories, but the present view of the overall pattern of reactions leading to the formation of fibrin is remarkably similar to the first proposed mechanisms<sup>75</sup>. The recognised factors involved in this cascade process have been allocated a Roman numeral by the International Committee on Haemostasis and Thrombosis<sup>90</sup> so as to standardise the nomenclature, though many are more commonly known by other names. Figure 2 shows the cascade scheme as presented by Esnouf and Figure 3 contains a table<sup>83</sup> listing the clotting factors along with some of their synonyms. This clotting cascade is in fact a biological amplifier, containing both positive and negative feedback loops: A small concentration of activated factor XII leads to ever increasing concentrations of the intermediate enzymes, yielding large quantities of thrombin<sup>75</sup>.

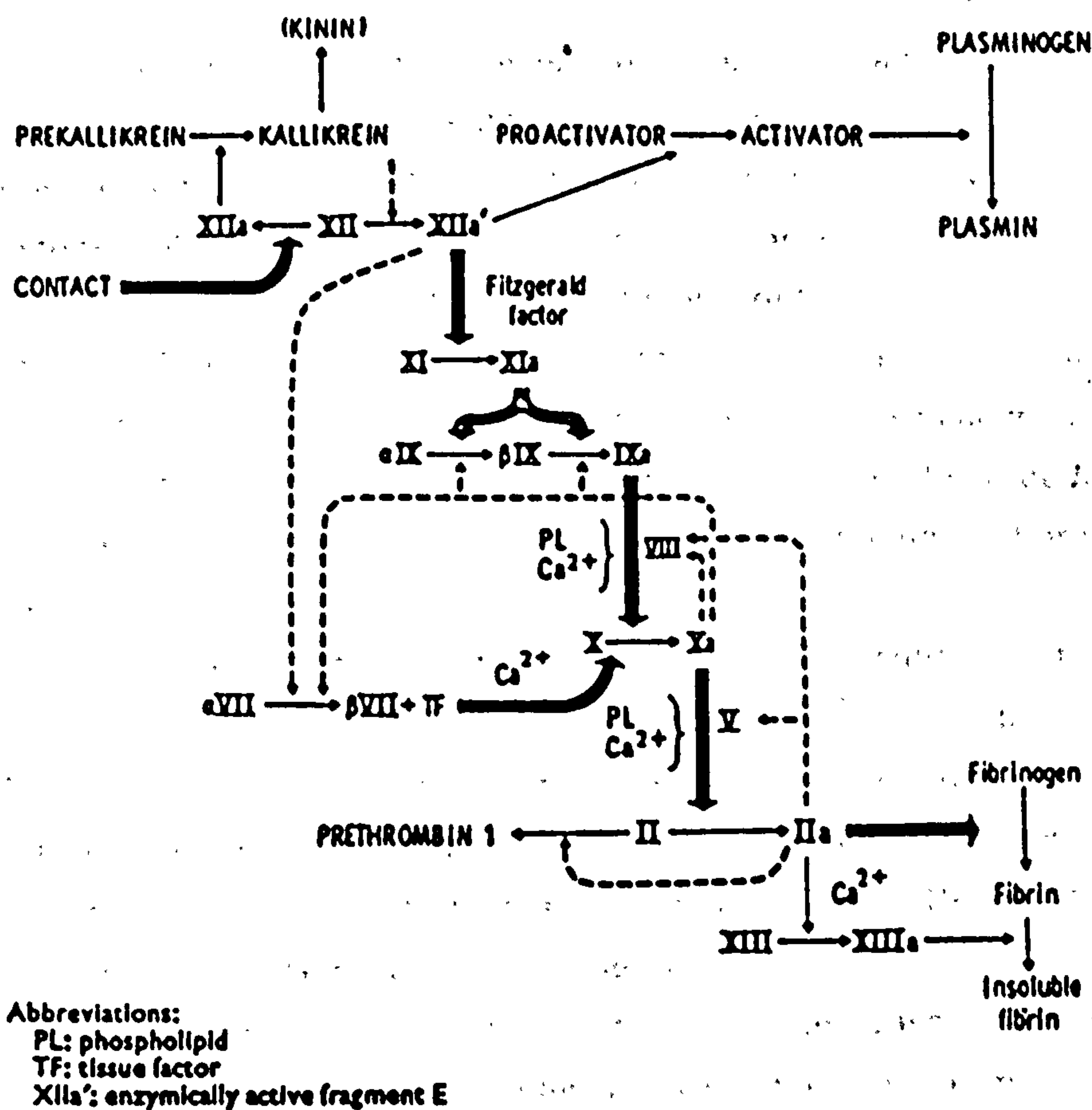


Fig. 2 A scheme for the interaction of the blood coagulation factors

Coagulation Factor		Coagulation Pathway			Molecular weight <sup>b</sup>
Roman numeral designation <sup>a</sup>	Common name	Intrinsic system	Extrinsic system		
Factor I	Fibrinogen	+	+		340,000 (human, bovine)
Factor II	Prothrombin	+	+		68,700 (human) 72,000 (bovine)
Factor III	Tissue factor		+		220,000; 330,000 (bovine)
Factor IV	Calcium ions	+	+		
Factor V	Proaccelerin	+	+		290,000-400,000 (bovine)
Factor VII	Proconvertin		+		63,000 (human)
Factor VIII	Antihemophilic factor	+			1.1 million (human, bovine)
Factor IX	Christmas factor	+			55,400 (bovine)
Factor X	Stuart factor	+	+		55,000 (bovine)
Factor XI	Plasma thromboplastin antecedent	+			160,000 (human, bovine)
Factor XII	Hageman factor	+			90,000 (human) 82,000 (bovine)
Factor XIII	Fibrin stabilizing factor	+	+		300,000 (bovine plasma) 320,000 (human plasma) 146,000 (human platelets)

<sup>a</sup> Activated factor V was originally thought to be a new clotting factor and was assigned Roman numeral VI.

<sup>b</sup> Some disagreement exists regarding the exact molecular weight of many of the coagulation factors, as noted in the text.

Fig. 3 Properties of some well-defined coagulation factors



This cascade mechanism is often viewed in three parts; an intrinsic and an extrinsic pathway leading to activated factor X and a common pathway covering the sequence of events initiated by this activated enzyme. The intrinsic system, only involving chemicals already present in blood plasma, is the means by which factor X is activated following contact of the blood with a foreign surface. The extrinsic system on the other hand, is initiated by chemicals such as tissue factor, released from the site of vascular injury. Both of these systems merge into a common pathway leading to the production of thrombin, which in turn catalyses the conversion of fibrinogen to fibrin which then polymerises into a mesh-like structure.

The initial aggregation of platelets into a plug and subsequent release reaction, whilst part of the normal clotting system, do not appear to be essential for haemostasis – at least in the case of canine blood. Cruz<sup>91</sup>, using a canine hind leg preparation, showed that the blood would clot in the absence of platelets. It is known, though, that substances such as ADP and factor III, released from platelets, greatly accelerate the clotting process<sup>92,93</sup>.

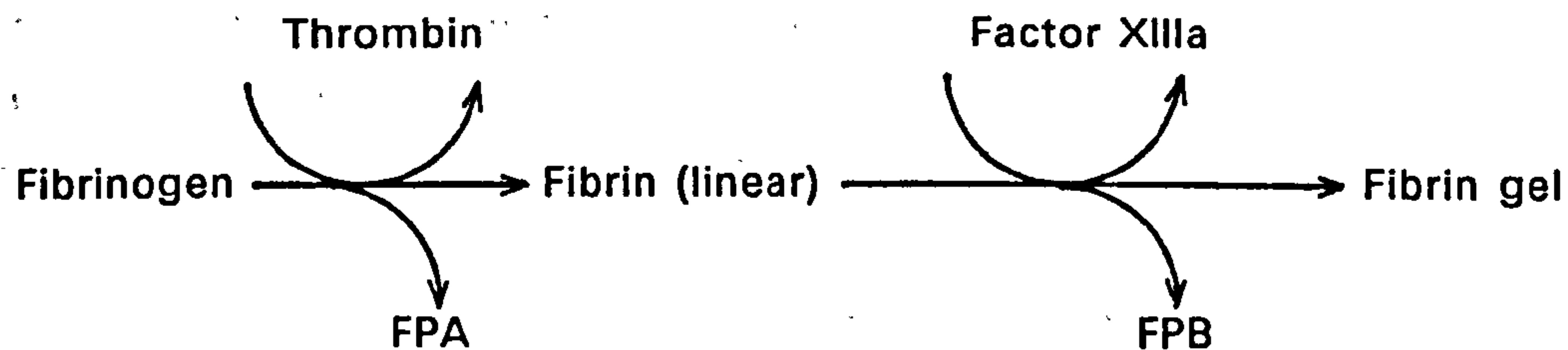
Under normal physiological conditions little or no intravascular coagulation occurs, even though clotting factors may be present arising from a localised injury. This absence of generalised clotting may be attributed to a number of effects<sup>83</sup>. In rapidly flowing blood dilution of the clotting factors will limit the concentration of factors that become activated. Thus, whilst general clotting may occur in disorders associated with venous stasis, a fibrin thrombus is most unlikely in the fast flowing arterial blood. Inhibitors of blood coagulation are present in the plasma deactivating coagulation enzymes such as thrombin or factor Xa. This, whilst being a slow process, is sufficient to remove small quantities of activated enzymes. Cells in the liver, known as Hepatocytes, remove proteins such as factor Xa, providing a purge and thus halting a general escalation in the concentration of activated enzymes present in the circulation.

In haemostasis, coagulation proceeds primarily through the extrinsic pathway, although chemicals released by the platelets and the endothelium serve also to initiate the intrinsic system. Thus both systems combine together *in vivo* to prevent bleeding. Absence of any of the clotting factors, e.g. factor XII in Hageman's Disease or factor VIII in Haemophilia, can greatly prolong the clotting process giving a high risk of haemorrhage<sup>94</sup>.

The final stage of the clotting reaction, that of the conversion of fibrinogen into a fibrin clot is thought to be a two-stage reaction<sup>89</sup>, involving the release of two fibrinopeptides, A and B. Fibrinopeptide A (FPA) is released first and a delay follows before the release of FPB, during which only linear polymerisation

of fibrin can occur<sup>88</sup>. Cleavage of FPB allows lateral polymerisation and the formation of a fibrin mesh.

The reaction can be summarised as follows:-



Calcium ions serve to activate factor XIIIa thus shortening the time for gel formation<sup>81,88</sup>. The observed sequence of events on adding thrombin to or activating the cascade mechanism in blood is thus a lag phase during which no clotted fibrin is visible, followed by a rapid second order gelation reaction.

The clotting time is known to depend on the initial concentration of prothrombin but the concentration of fibrinogen is apparently not rate determining. The clotting time,  $t$ , is found to be dependent on the prothrombin concentration,  $c$ , according to the following equation<sup>95</sup>.

$$t = a / c + b$$

where  $a$  and  $b$  are constants.

Lee and White<sup>94</sup>, in 1913, devised what is now a standard test for clotting time. 1cm<sup>3</sup> of blood is drawn from a vein in the arm into a glass syringe, previously rinsed in saline. Noting the time, the blood is transferred to a glass test-tube (Widal tube) of 8mm diameter (also rinsed in saline). The test tube is inverted every 30 seconds until the blood is found to clot, i.e. the blood no longer flows from one end of the tube to the other. The time at which this happens is recorded and the clotting time is taken as the duration of the experiment. The normal blood coagulation time is about 6.5 minutes for a healthy person, although for haemophiliacs it may be as high as 55 minutes and in Brill's disease as low as 2 minutes.

## 2.2 Thrombosis

Thrombosis is the clotting process of flowing blood, usually in contact with a foreign surface<sup>96</sup>. Before any formed elements of the blood are observed to adhere to the surface a protein layer is thought to deposit<sup>97-100</sup>. This is normally followed by the adhesion of a monolayer of platelets, a process that is believed to occur at a rate independent of the properties of the surface<sup>48</sup>. With some 'inert' surfaces, under mild flow conditions, no further activity



occurs<sup>101</sup>. These inert surfaces, e.g. Carbon, are thought to absorb the protein layer but not to alter its structure, thus inhibiting platelet adhesion<sup>60</sup>.

The next process appears to be the formation of pseudopodia by the adhering platelets and the migration of these platelets into aggregate lumps around surface imperfections (These aggregates become widespread if the surface is inherently thrombogenic.)<sup>101</sup>. The deformed platelets release chemicals into the blood: ADP, platelet factor 3, serotonin, adrenalin, fibrinogen, etc.. ADP in particular is known to cause further platelet aggregation, reversible with low ADP concentrations but irreversible in high concentrations<sup>102</sup>.

Further aggregation of platelets will only occur if the local concentration of activating species is above a threshold value and if platelets are carried to the surface in sufficient numbers<sup>66,99</sup>. If either of these conditions is not met then aggregate growth stops. The thrombi formed are not static structures: Baumgartner<sup>103</sup> has shown that removal of the endothelium of a rabbit's aorta results in immediate formation of platelet thrombi on the subendothelium, but that 40 minutes later only a thin layer of platelets remains. The behaviour of the platelets, dislodged from such aggregates, on returning to the circulation, was ascertained by testing their ability to aggregate in the presence of either ADP or thrombin. No significant differences were observed between these platelets and normal healthy platelets.

If regions of stasis develop between the aggregates, chemicals released from the aggregated platelets may trigger the cascade of reactions leading to the formation of fibrin. This fibrin polymerises into a mesh around the platelet aggregate, thus giving stability to the thrombus clot<sup>102</sup>. On heparinised surfaces this mesh will not form, presumably due to inhibition of thrombin production by the heparin<sup>98</sup>.

The type of thrombus formed is dependent on the shear rate and the properties of the material in contact with the blood, ranging from a monolayer of platelets on inert materials under favourable hydrodynamic conditions, through platelet aggregates, with or without the stability offered by a fibrin mesh, in regions of high shear to a normal fibrin mesh containing erythrocytes, leucocytes and platelets in regions of low shear. In flowing blood it is therefore usual to find that the upstream edge of a thrombus is composed primarily of platelets in a fibrin mesh with the concentration of larger corpuscles increasing progressively downstream<sup>67,97,104</sup>.

Whilst the chemical sequence of events has not been investigated in flowing blood there seems no reason to doubt that the final fibrin clot is a result of activation of the intrinsic, and possibly the extrinsic, clotting pathway. Thrombosis will not lead to a universal coagulation of the blood for the same

reasons as were proposed regarding haemostasis, i.e. dilution, chemical deactivation and removal of activated clotting factors in the liver.

Fibrinolysis, whilst a useful mechanism in haemostasis where healing of the wound requires that the clot should slowly disintegrate, making way for new tissue, can be dangerous in thrombosis by weakening the adhesion of the thrombus to the surface and thus enabling the release of an embolus into the bloodstream. If this thromboembolus is not removed or destroyed it may cause a stroke by blocking one of the small cranial arteries<sup>102</sup>.

Another phenomenon, known to occur in the stagnation region of a jet of fluid impinging on a surface, is that of white blood corpuscle deposition. A white cell circle forms, the circumference of which is thought to be shear rate limited. Petschek quotes a shear rate of  $5.5 \text{ s}^{-1}$  above which white cells will not adhere to the foreign surface<sup>67</sup>. With very low flows where the white cell circle is able to exceed the diameter of the jet, as the surface shear rate does not exceed  $5.5 \text{ s}^{-1}$ , a more general platelet thrombus is observed to form within the white cell circle.

### **2.3 Haemolysis and Platelet Damage**

Whilst the clotting of blood and haemolysis (the breakdown of red corpuscles) occur for totally different reasons, they are often related. In the latter stages of the formation of a blood clot, red blood cells, caught in the fibrin mesh are known to release their contents, which in turn promotes thrombus growth<sup>104</sup>. Haemolysis too can lead to clotting, depending on the severity of the haemolytic process<sup>85,105,106</sup>. With haemolysis induced by prosthetic cardiac valves the outcome of the release reaction will largely depend on whether the components are swept away from the surface as rapidly as they are exuded. In most cases the released components, capable of activating the thrombotic chain of events, are diluted or removed before platelets or fibrin adhere to the foreign surface, since haemolysis generally occurs in fast flowing blood. However if the released components enter an area of stasis in the vicinity of the valve's surface, these chemicals, especially ADP, ATP and erythrocytin, may result in platelet adhesion and activation of the coagulation process. In this respect haemolysis in the case of multiple implants is more serious than with single valve replacement, as haemolysis at the first of the implants may lead to enzymes collecting in regions of stasis around the second implant.

Platelet damage<sup>107</sup> also releases chemicals (ADP, ATP, factor III, serotonin, Adrenalin, Lysosomal enzymes, cationic proteins, mucopolysaccharides, fibrinogen and platelet factor 4)<sup>92,93,102</sup> which will lead to increased adhesion of platelets and stimulus of the coagulation process<sup>108,109</sup>. Again this is only thought to be serious if the concentration of these components is allowed to build up near a foreign surface.



## **Chapter 3. Fluid Mechanical Factors Relating to Thrombosis**

### **3.1 Possible Fluid Mechanical Determinants of Thrombosis**

A number of fluid mechanical phenomena are thought to be associated with thrombosis: Elevated shear stresses<sup>107</sup>, turbulence<sup>65</sup>, flow impingement on a surface<sup>66</sup>, stasis, surface contact time and elevated rates of mass transfer<sup>110</sup>.

Shear is unlikely to be directly related to the deposition of clot on a surface, except in so far as elevated shear stresses can damage either the formed elements of the blood<sup>105,107</sup> or the endothelial lining of the vessel<sup>111</sup>. Such damage would cause the release of coagulation inducing enzymes into the plasma<sup>112,113</sup>. However shear may also denature some of the proteins involved in the clotting reaction. Katoh<sup>114</sup> et al have shown that shear can reduce the rate of activation of factor X via the intrinsic pathway, and fibrinogen is also known to be susceptible to shear-related damage.

High shear rates are normally associated with high rates of mass transfer. This mass transfer again has a two-fold influence: By causing a high rate of transfer of platelets to the surface, aggregates can form and grow rapidly (assuming that this aggregation process is diffusion-limited), but by causing a high transfer rate of aggregation-inducing enzymes away from their point of release at the surface a reduction of the aggregation rate will ensue due to dilution. A further observation of the behaviour of particles in shear is pertinent. Mashelkar<sup>115</sup> and Saffman<sup>116</sup> have predicted that in steady shear macromolecules and spherical particles will migrate away from regions of high shear. The forces encountered in normal shear fields are small, as are the consequent movements of particles. However, in regions of high shear and accelerating flow, where suspended particles such as red blood cells may lag behind the fluid motion, Saffman's forces may contribute significantly to particle motion. Since shearing forces are normally highest next to a surface, shear induced mass transfer will, if anything, be reduced by such effects. Wall shear may also inhibit the deposition of formed elements, tearing off aggregated platelets if the aggregation forces are weaker than the shearing forces.

Shear stress is therefore perceived to be capable of both enhancing and inhibiting platelet deposition. Its main contribution to thrombosis may be that of rendering the blood hypercoagulable by causing release of chemicals from formed elements in the blood, rather than by enhancing local deposition.

Flow impingement on a surface can also lead to high transfer rates of material to the surface. However, as with regions of high shear parallel to a surface, fluid is also washed away rapidly from such regions and unless the

impinging blood is already activated, so that platelet aggregation can occur on contact, such sites are unlikely to be favoured for deposition. Forcible contact may however damage the blood elements, potentially causing coagulation elsewhere.

Turbulence leads both to increased levels of shear within the bulk fluid and to increased rates of material transfer to the surface, and so presumably will lead to a combination of the factors mentioned above.

Regions of stasis are areas where the fluid remains for periods in excess of the mean residence time of the bulk fluid. These may be associated with recirculating fluid or with stagnant, 'deadwater' regions. In either case, mass transfer to or from such regions is generally slow in relation to that occurring elsewhere in the fluid. Thus if activated, or activating, species enter, or are released, in such a region they may remain there for a significant period of time. If a foreign surface is also present, such as is the case with stasis caused by the presence of a prosthetic heart valve, then prolonged contact of the blood with the material may result in platelet<sup>117</sup> and/or fibrin deposition.

Regions of stagnation or stasis may therefore be associated with thrombosis if mechanisms exist for introducing activated coagulating species into such regions, and if a suitable surface is present on which the thrombus can form. In a recirculating vortex some fluid enters the vortex at the point of flow reattachment to the fluid boundary. Since this is a stagnation point where the flow impinges on the surface, it is possible that species activated at this point may then enter the vortex. Also a small amount of particle, or protein, migration may occur across the boundary between the fast-moving fluid outside the vortex and the slow-moving fluid inside. This migration may result from Saffman forces or through bulk fluid movement in the form of small eddies resulting from flow instabilities at the vortex boundary.

In this chapter the experiments performed to investigate the fluid-mechanical causes of thrombosis are reviewed and the current position regarding the influence of each flow-characteristic discussed. Two types of experiment have been carried out to investigate fluid mechanical influences of thrombosis. The first has been to observe clot deposition in various blood flow regimes, usually of fairly simple geometry, where some aspects of the nature of the flow are known. The second has been to analyse the detailed structure of the flow *in vitro* in similar flow geometries to those in which blood is known to clot in clinical practice, and in particular to scrutinise the flow patterns observed in the vicinity of artificial heart valves where thrombus is known to deposit. Some overlap exists between these approaches, where experimenters have studied both thrombosis and flow characteristics in the same, or similar, apparatus.



## 3.2 Experimental Investigations of Thrombosis

### 3.2.1 *In Vivo*

A number of *in vivo* experiments have been conducted, mainly in dogs, to investigate hydrodynamic aspects of thrombosis. Many of these experiments were designed initially as compatibility tests for materials with blood, with analysis of the influence of flow being an afterthought.

Gott, having successfully tested materials by insertion of smooth tubes<sup>52</sup>, coated with the material under test, in the canine vena cava, progressed to inserting 'swords' of rigid material and 'flags' of flexible material into the canine right atrium<sup>53</sup>. This allowed for the testing of four materials simultaneously, rather than just one as in his original test, thus reducing the number of animal tests considerably. The results proved to be less consistent than those of the vena caval ring tests. The authors accounted for this inconsistency by suggesting that it was due to the unpredictable fibrinolytic activity in the experimental animal: A significant thrombus, present after four hours, may be completely lysed after 24 hours. Fibrinolysis does not appear to be such a critical factor in the vena caval ring test<sup>62</sup>. Differences in fibrinolytic activity between the animals may be the cause of the inconsistency in the right atrium, but that in itself does not explain why the vena caval test is more consistent. Two further observations may serve to explain why variations in fibrinolytic activity may be more critical in the chambers of the heart. Firstly it is possible that movement of the atrial wall during the pumping cycle may assist in dislodging clot from the wall under the action of fibrinolysis. In the vena caval ring test thrombus forms in the rigid tube and will thus be protected from mechanical dislodgement. Secondly, flow recirculation in the atrium may result in prolonged residence times for some of the fluid in this region so that fibrinolytic enzymes may possibly become concentrated in the region of the clot (presuming that these enzymes are released from trapped or aggregated particles), whereas in the vena cava a relatively steady flow of blood exists which may wash away any fibrinolytic enzymes released.

Seidel<sup>118</sup> also investigated the effects of insertion of objects into the canine heart, using both the atrium and the ventricle. By suspending strips of rigid material, using a wire framework, between the right atrium and ventricle he observed that clot propagated along the material away from the point of attachment to the endothelium. This thrombus was presumably initiated by damage at these sites. When the material was replaced by a flexible plastic, or was suspended from the endothelium by threads instead of wire, clot was not observed to propagate, although initiation of clot at the damaged endothelial wall still occurred. A silicone rubber ball was then suspended by a thread in the right ventricle. Thrombus covered the ball after a few days, starting at the

point of attachment of the thread to the ball and proceeding to grow until the whole ball was coated. This clot did not appear to adhere directly to the surface of the ball, presumably using the thread as an anchorage.

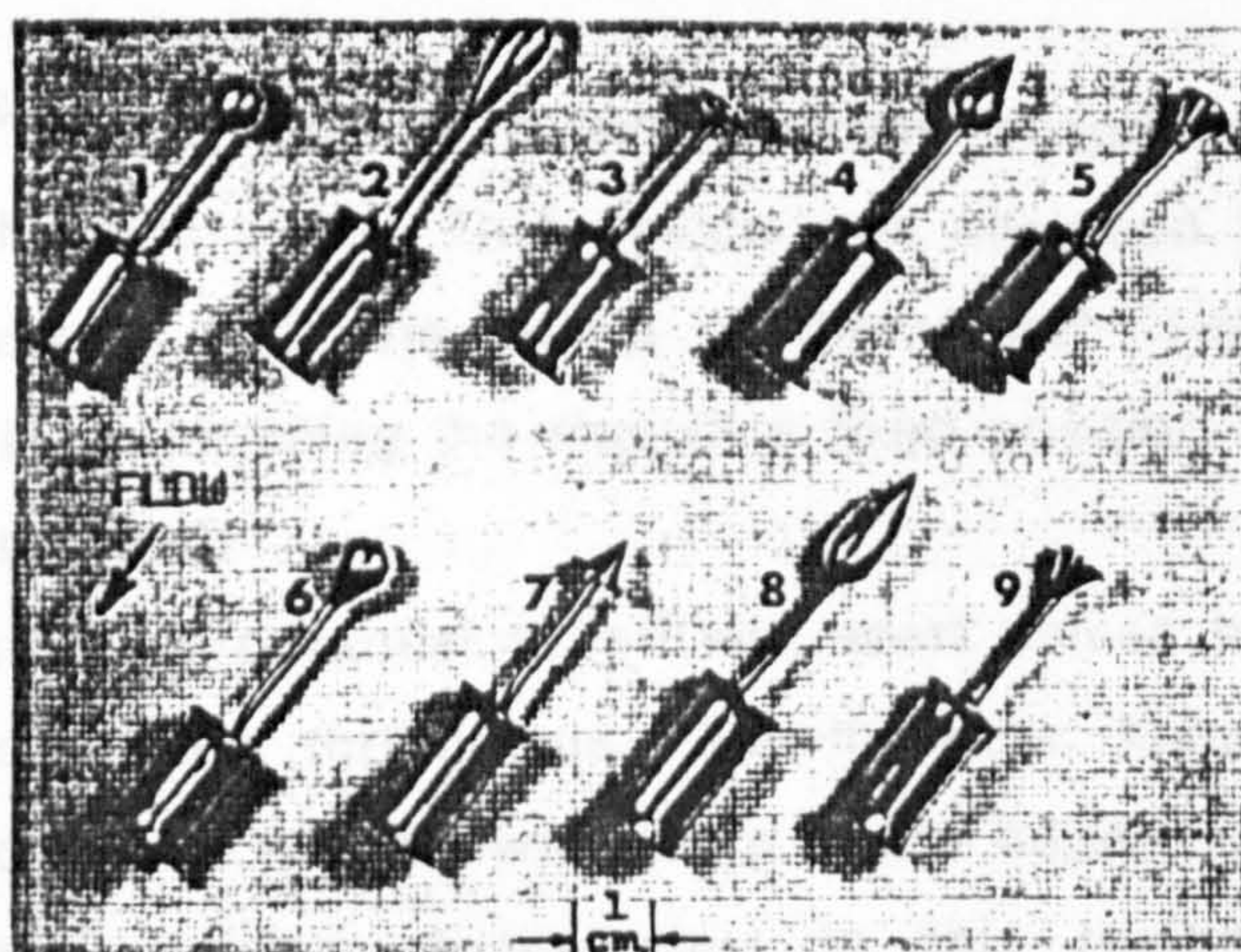
These cardiac experiments of Gott and Seidel yield little clear information from a hydrodynamic point of view since the flow within the heart chambers is complex and the movement of these implants during the cardiac cycle is unknown. The *in vivo* experiments, specifically designed to yield information on the flow dependence of thrombosis, have generally involved insertion of axisymmetric test bodies into the large arteries or veins, or the use of arterio-venous shunts. Examples of the former include Gott's vena caval rings<sup>52</sup>, Kusserow's renal test rings<sup>54</sup>, Vorhauer's aortic test objects<sup>65</sup> and Lederman's magnetically suspended slug<sup>55</sup>.

Gott<sup>62</sup> discovered that insertion of a centrally-positioned, square-edged orifice into his original vena caval rings had no influence on thrombosis with materials found to remain thrombus-free in the original test, unless the orifice diameter was reduced below 6mm (The internal diameter of the empty tubes being 7mm). With a 5.8mm orifice, however, thrombus would form, primarily on the upstream face of the orifice. Fairing of this face with a 1mm radius of curvature rendered the test piece athrombogenic once more. On reducing the orifice diameter to 5mm, thrombus would form regardless of whether the upstream face was faired. It was discovered however, that the combination of positioning the orifice at the entrance of the tube and fairing the upstream face once again resulted in the test piece remaining thrombus-free. Blackshear<sup>63</sup> showed that in the cases where the thrombus is observed to form, the site of flow reattachment downstream of the orifice (predicted by an *in vitro* flow experiment) is expected to be downstream of the trailing edge of the inserted sleeve. He postulated that thromboplastins, released from damaged tissue at the site of anchorage of the test-sleeve, would thus be trapped in this vortex downstream of the orifice, rendering the blood hypercoagulable in this region. In the case where flow reattachment occurs within the sleeve these thromboplastins would be washed downstream, diluted and destroyed. This explanation, however, whilst compelling, is not convincing since coagulation is observed to begin through the formation of a platelet thrombus in the region of flow separation on the leading edge of the orifice, where the state of the blood trapped in the downstream vortex has no influence. Further investigation of the growth of thrombus in these rings would be necessary to determine whether thrombus, in the case of the faired rings, only occurs downstream of the orifice, in which case Blackshear's analysis may well apply for such downstream clotting.



Kusserow<sup>54</sup>, in an attempt to measure the total clot (i.e. both clot adhering to, and emboli dislodged from, the surface) formed by the insertion of an arterial ring implant, inserted rings in the canine aorta immediately upstream of the origin of the renal system. By subtotal aortic constriction below this origin of the renal vessels it was hoped to trap emboli in the kidneys. The results proved inconclusive, with renal infarcts occurring even when the test ring remained thrombus-free.

Davila<sup>119</sup> et al report the insertion of pins of various cross-section into the canine aorta. A layer of clot formed, encapsulating the triangular and circular pins, but only a streamer of clot was found behind elliptical pins. A much more controlled and systematic investigation has been carried out by Vorhauer<sup>120</sup>. Vorhauer implanted nine different bodies of revolution, machined in aluminium and coated with polyurethane, in the canine descending thoracic aorta. In the first set of 108 tests (testing three objects sequentially in each dog), each of 45 minutes duration, the objects were mounted on a sting at their downstream end (Fig. 4 ). In these experiments only clot adherent to the objects at the end of the experiment was weighed. Clot was observed to form in the wake of the objects, the quantity of which appeared to be related to both the turbulence intensity and size of the wake<sup>121</sup>. The extent of red corpuscle aggregation within the thrombus decreased as the turbulence intensity increased.

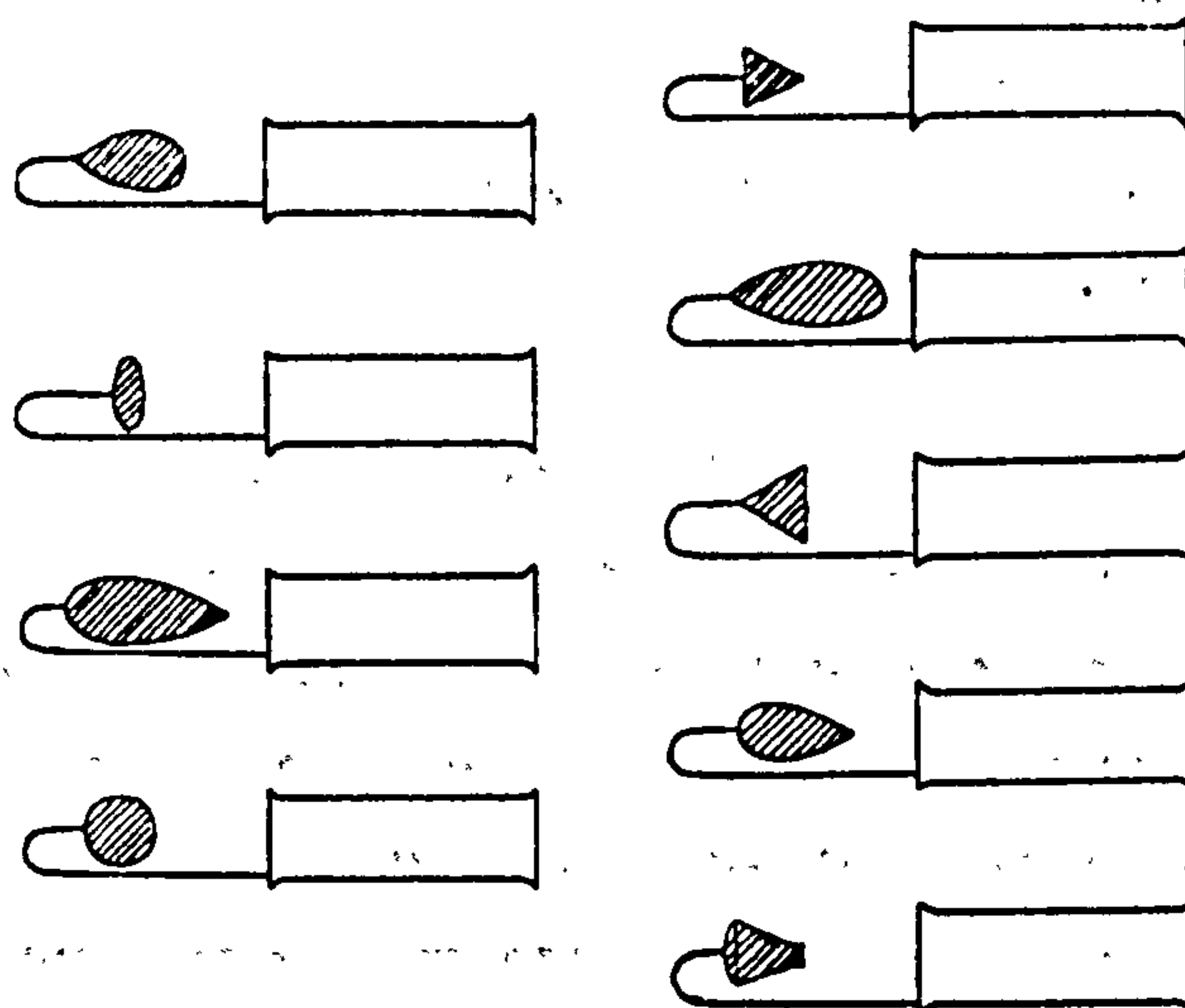


**Fig. 4** Vorhauer's first set of mounted test bodies

In a second set of experiments to determine the influence of turbulence downstream of the objects, the test-bodies were mounted through their upstream faces (Fig. 5 ). After 20 minutes the clot downstream in the mounting tube alone was weighed. An unexpected phenomenon occurred: The mounting ring, without a test body attached, accumulated a larger quantity of coagulum than all but one of the rings with test bodies in place. Vorhauer also observed flow patterns by means of a birefringent fluid and attempted to determine turbulence energy spectra from wall pressure measurements for the



same objects in a mock circulation rig. Whilst the wake turbulence immediately downstream of the objects varied as expected, with the bluff bodies generating more turbulence than the streamlined ones, the wall turbulence on the mounting tube, when averaged over the pulsatile cycle, was lower in a number of cases with objects present than that for the empty tube. No clear pattern emerges, however, between the ordering of the objects on the basis of wall turbulence intensities and that on the basis of clotting in the mounting sleeve.



**Fig. 5** Vorhauer's second set of mounted test bodies

Lederman<sup>55</sup> made use of a magnetically suspended slug in the canine descending thoracic aorta. This device was designed to avoid contact of the implanted material with the intima of the vessel. However, difficulties were encountered in maintaining the slug in a fixed position and the idea appears to have been discarded.

Animal testing of potential prosthetic heart valves has also revealed useful information regarding thrombosis. For instance, Knight<sup>122</sup>, in early trials of pivoting disc valves has shown that a sharp edged sewing ring will not only lead to large amounts of thrombus in this region, but will also cause thrombosis on the valve where the jet caused by the sewing ring orifice impinges on the tilting disc. The use of a streamlined disc rather than a flat one reduced the incidence of thrombus on its trailing edge. In addition to these planned *in vivo* experiments, a large body of information has been obtained from the clinical use of prosthetic valves. Thrombus tends to form with the Starr-Edwards valve around the sewing ring and at the apex of the struts, with the Bjork-Shiley valve in the region of the minor orifice<sup>11,80</sup> and with the St. Jude Medical valve in the deadwater region downstream of the leaflets<sup>40,41</sup>.

### 3.2.2 Ex Vivo

A number of *ex vivo* experiments have been performed in which blood is drawn directly from the animal's vascular system into the experimental flow chamber. The blood is then either returned to the animal or discarded. Where the blood is returned to the animal, the test chamber is normally an arterio-venous shunt.

Several arterio-venous shunts have been designed, mainly for testing the influence of turbulence on the clotting of blood. These are essentially extracorporeal systems but since they use blood direct from the animal it is expected that coagulation occurring within them will be comparable to that *in vivo*. This is not unreasonable in that an arterio-venous shunt is not significantly different from the vena caval ring, except in relation to its length and the use of arterial rather than venous blood.

Smith<sup>123</sup> et al established, in each of six dogs, two arterio-venous shunts from a femoral artery to the contra-lateral femoral vein, one containing a 'turbulence producing device', the other not. The shunts were otherwise identical in size, shape and material. The turbulence generator (Fig. 6) consisted of a 4.8mm diameter smooth tube with an orifice of 1.6mm diameter, having a tapered upstream face. The flowrates through the pairs of shunts, measured using electromagnetic flowmeters, were kept equal by means of a screw clamp on the tubing to the laminar flow shunt. When the flowrate fell such that the Reynolds number in the turbulent shunt dropped below 700 the experiment was stopped and the shunts weighed. The weight of thrombus deposited in the 'turbulent' shunt was 3.4 times that in the laminar flow shunt when measured whilst wet and 5.3 times heavier when dry, showing that not only does turbulence increase the amount of deposited thrombus, but that it also leads to a more densely packed clot structure.

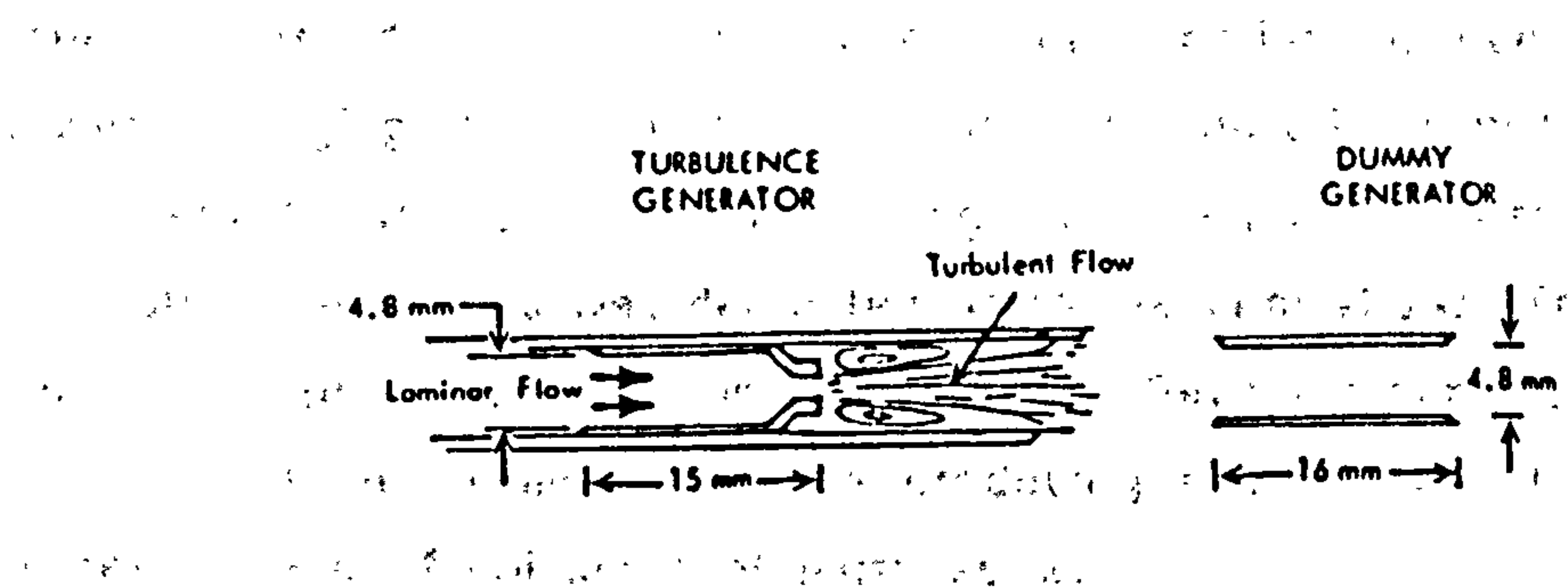


Fig. 6 Smith's Turbulence Generator

Stein and Sabbah<sup>124</sup> carried out a similar study, again establishing two arterio-venous shunts in similar positions in each of eight dogs. Again, apart from the presence of a turbulence producing device, this time a 1.6mm diameter orifice tapered at both ends (Fig. 7), the two shunts were identical.



The wet weight of thrombus produced in their experiments in the turbulent-flow shunt was 200 times that in the laminar-flow shunt.

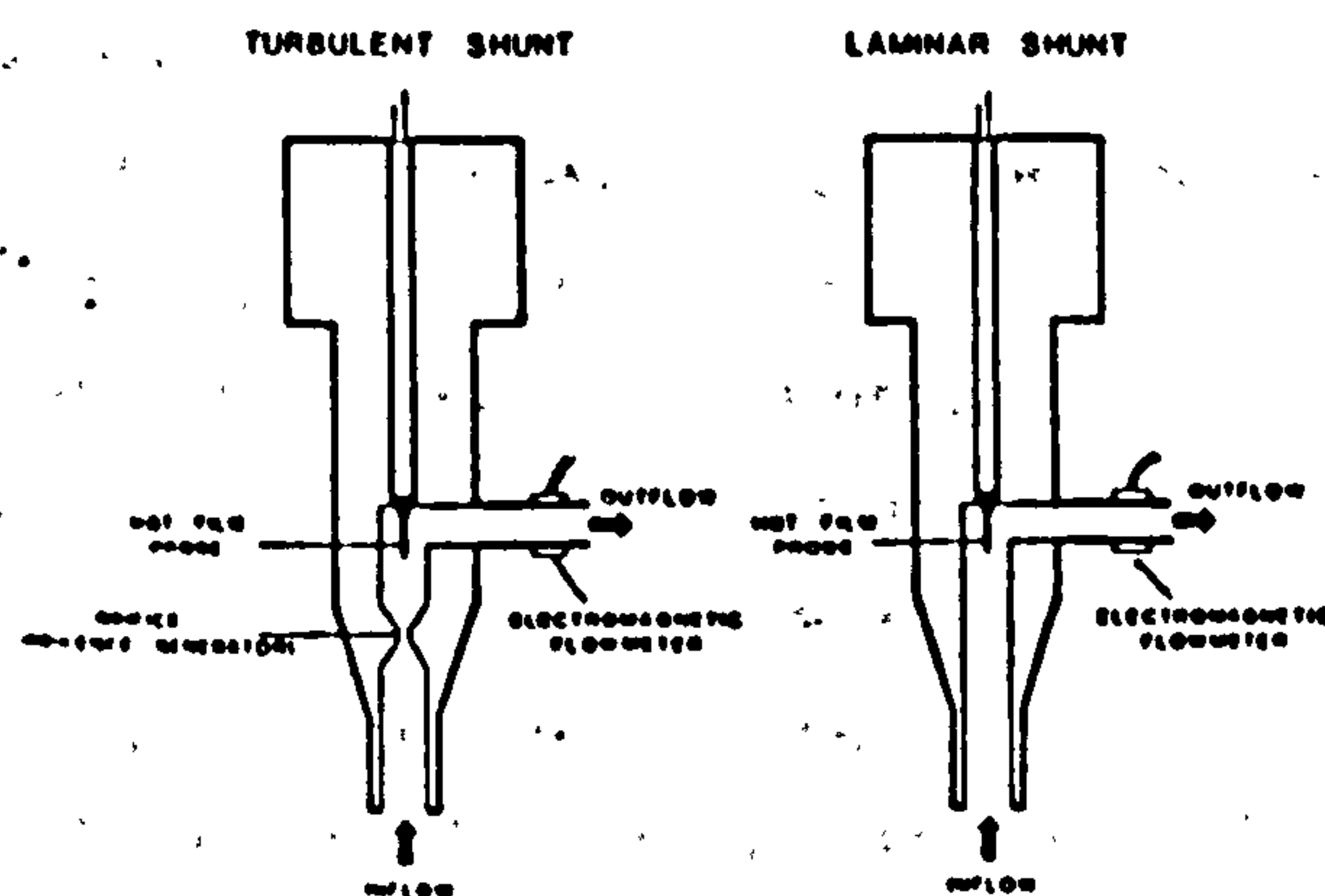


Fig. 7 Stein's Turbulence Generator

Stein and Sabbah's experiments differed from Smith's in one major respect. Smith et al had allowed the experiment to run until the flow in the shunts had dropped such that the turbulent shunt Reynolds number had fallen to about 700. Thus in their experiments the Reynolds numbers ranged from 2800 to 350, decreasing over the course of the test. The duration of the experiments was not recorded. The average wet weight of thrombus was 4.9g for the turbulent shunt and 1.4g for the laminar shunt. Stein and Sabbah, on the other hand, whilst using a fairly similar turbulence promoting device, restricted the duration of their experiments to 7 minutes so that the Reynolds numbers remained almost constant over the course of the experiment, implying a constant flow regime throughout. They carried out several experiments in each dog at different Reynolds numbers to deduce the effect of Reynolds number (and thus turbulence) on the quantity of clot formed. Since the experiments were of much shorter duration the wet weights of thrombus formed were also much lower, averaging 180mg in the turbulent shunt and 0.9 mg in the laminar shunt. This value for the laminar shunt is slightly misleading in that in 17 out of the 21 tests no thrombus was deposited on the laminar shunt. Their results show that the weight of thrombus increases as the Reynolds number increases in the turbulent shunt. Their turbulence producing device leads to high shear, an increased rate of collisions of particles with the wall and possibly prolonged contact of blood with the wall in the region immediately downstream of the orifice, if a vortex is present, though tapering of the trailing edge of the orifice should minimise this effect (In dye experiments downstream of the orifice in a mock circulation Stein and Sabbah observed that dye enters and leaves the recirculating region rapidly thus showing that prolonged stasis does not occur.). Stein and Sabbah also measured turbulence intensities downstream of the



orifice by hot film anemometry in the mock circulatory loop. The turbulence intensity was found to increase almost linearly with Reynolds number, as did the thrombus weight. They therefore relate the degree of thrombosis to the relative turbulence intensity.

Leonard<sup>125</sup> has investigated the use of an arterio-venous shunt containing a square edged circumferential groove. Thrombosis was found to occur within the recirculating fluid trapped in the groove, especially in the recessed corners. No thrombus was deposited from the laminar blood flow in the main tube. Stasis within the groove is proposed as the cause of this thrombus.

Perhaps the most notable of the *ex vivo* experiments are those using Petschek's Stagnation Point Flow Chamber<sup>56</sup>. The flow chamber (Fig. 8) comprises a raised flat central region surrounded by a circular trough, whose outside walls are higher than the raised region. Over the top of this cavity a microscope cover-slip is placed, coated on the lower side with a suitable biomaterial (The test has indeed been used for testing the blood compatibility of such biomaterials<sup>126</sup>). Blood from a cannulated canine carotid artery enters the test chamber through a centrally located hole in the raised region, impinging perpendicularly on the cover-slip. The blood then flows radially outwards through the annulus formed by the cover-slip and the raised plateau, collecting in the trough from which it is metered out steadily via a withdrawal port.

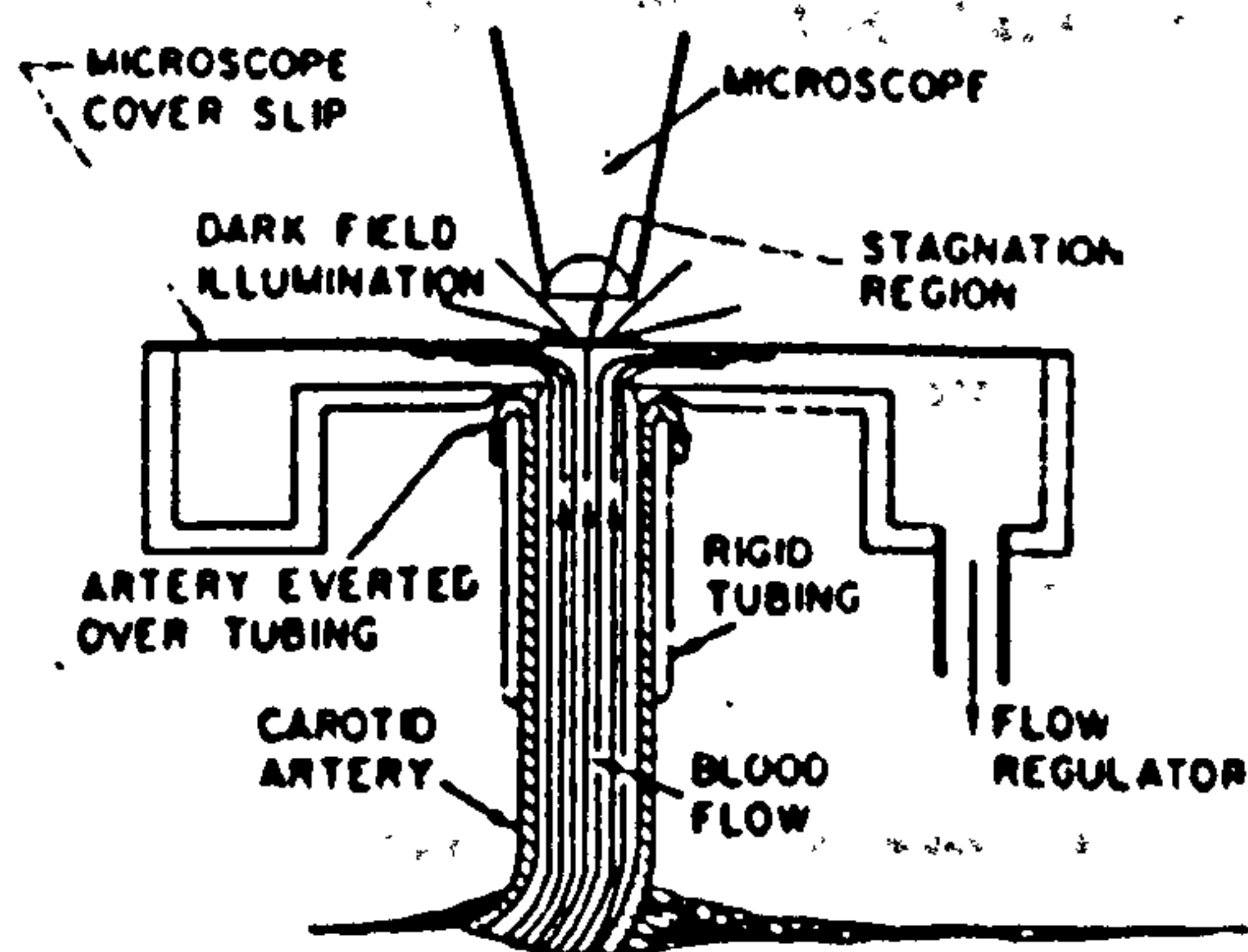


Fig. 8 Diagram of Petschek's Stagnation Point Flow Chamber

The point where the flow impinges on the cover-slip, a stagnation region, is observed microscopically under dark field illumination. A blood flowrate of 2ml/min was used so that even in an experiment of 3 hours duration only a fifth of the total blood volume of the dog will be used. Seven flow chambers of five different dimensions were used. The wall shear stress,  $\tau$ , in the stagnation region rises linearly with the radius,  $r$ , from the centre of the zone according to the relation:  $\tau = \beta \mu r$ , where  $\beta$  is a constant depending on the flow rate and the viscosity of the blood, forming a vortex around the

where the flow parameter,  $\beta$ , is a property of the width of the annular region and the diameter of the entrance hole, and  $\mu$  is the dynamic viscosity of the fluid. In Petschek's experimental chambers  $\beta$  values of 1.9, 8.6, 17, 157 and  $200 \text{ mm}^{-1} \text{ s}^{-1}$  were used.

Using this type of chamber, Petschek et al have observed the sequence of events leading to thrombus formation on a foreign surface<sup>67,97,104</sup>. These are summarised in figure 9. The first stage is assumed to be the absorption of a protein layer<sup>67</sup>. This is not visible under the microscope but is proposed as an explanation for the time lag of 1/2 to 1 minute during which no formed blood elements adhere to the surface. The rate at which platelets adhere to the surface depends predominantly on the flux of platelets to the surface<sup>101,126</sup> and possibly also on the surface properties of the material<sup>51</sup>. At high flow rates, and therefore under high shearing rates, on relatively athrombogenic surfaces this layer of deposited platelets remains intact<sup>97</sup>. The only thrombi that form on such surfaces are wedge-shaped thrombi<sup>66,97</sup>. The wedge thrombi<sup>67,104</sup> that form in these high-flow experiments appear to be initiated by the presence of surface imperfections. They grow rapidly on their downstream side spreading out in the process. They also grow slowly on their upstream face. It is thought that thrombus growth is due to released material from the already aggregated platelets diffusing outwards. These materials will collect preferentially in the wake region where diffusion will be aided by the flow and where the exchange of fluid with the bulk is slow, leading to accelerated growth there.

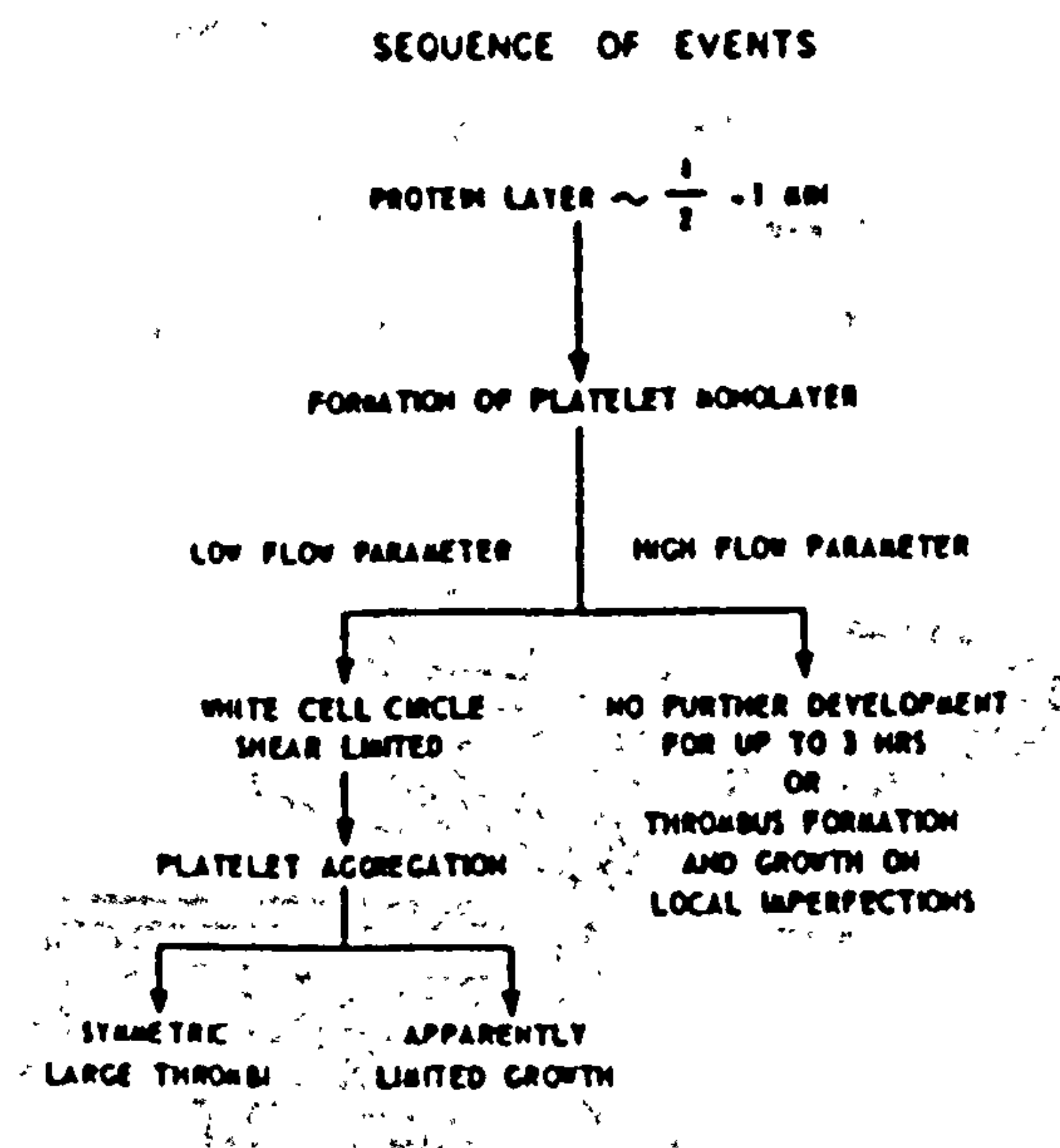


Fig. 9 Sequence of events leading to thrombus formation

Where the surface only exhibits moderate thromboresistance or when the flowrate is low, white cells adhere to the surface, forming a circle around the



stagnation point, the diameter of which appears to be shear-rate limited<sup>67,126</sup> with no white cells depositing where the wall shear exceeds  $0.6\text{s}^{-1}$ .

Under extremely low flowrate conditions, or on thrombogenic surfaces, white cells may adhere over the whole surface. Subsequently large platelet aggregates may form in a symmetrical pattern around the stagnation point<sup>67</sup>. These symmetric thrombi are thought to occur as a result of the inherent thrombogenic characteristics of the test surface, in that they do not initiate at any particular location in the flow field<sup>97</sup>.

Morton<sup>127</sup> et al also report the inclusion of a forward-facing step in the test chamber. With this flow configuration platelet aggregation was always observed distal to the step. Within the separated flow in this region, freely floating platelet aggregates were observed to form and grow. Occasionally these aggregates, up to  $30\text{--}45\mu\text{m}$  in diameter, would escape from this separated flow region. Morton pointed out that this type of behaviour could lead to the release of emboli into the blood flow.

Dutton<sup>98</sup> has carried out a similar set of experiments to those of Petschek, using a semicylindrical test section, again with a stagnation zone where the blood, entering via a hole in the cylindrical surface, impinges on the flat cover-slip (Fig. 10). The blood in this case is drawn from the canine jugular vein at a constant rate of  $1.94\text{ml/min}$  through each end of the hemicylinder by means of a double syringe pump, thus keeping the wall shear at  $60\text{s}^{-1}$  over most of the flat surface. The events in the stagnation region are observed using reflected light microscopy. At the end of each experiment the thrombus formed is examined under a microscope. The experimental duration in some experiments is limited to two minutes, after which the cover-slips are removed, rinsed in saline and then viewed under a microscope. In these experiments platelets were seen to be randomly scattered over the whole surface except in regions where contamination of the surface led to the growth of thrombus wedges.

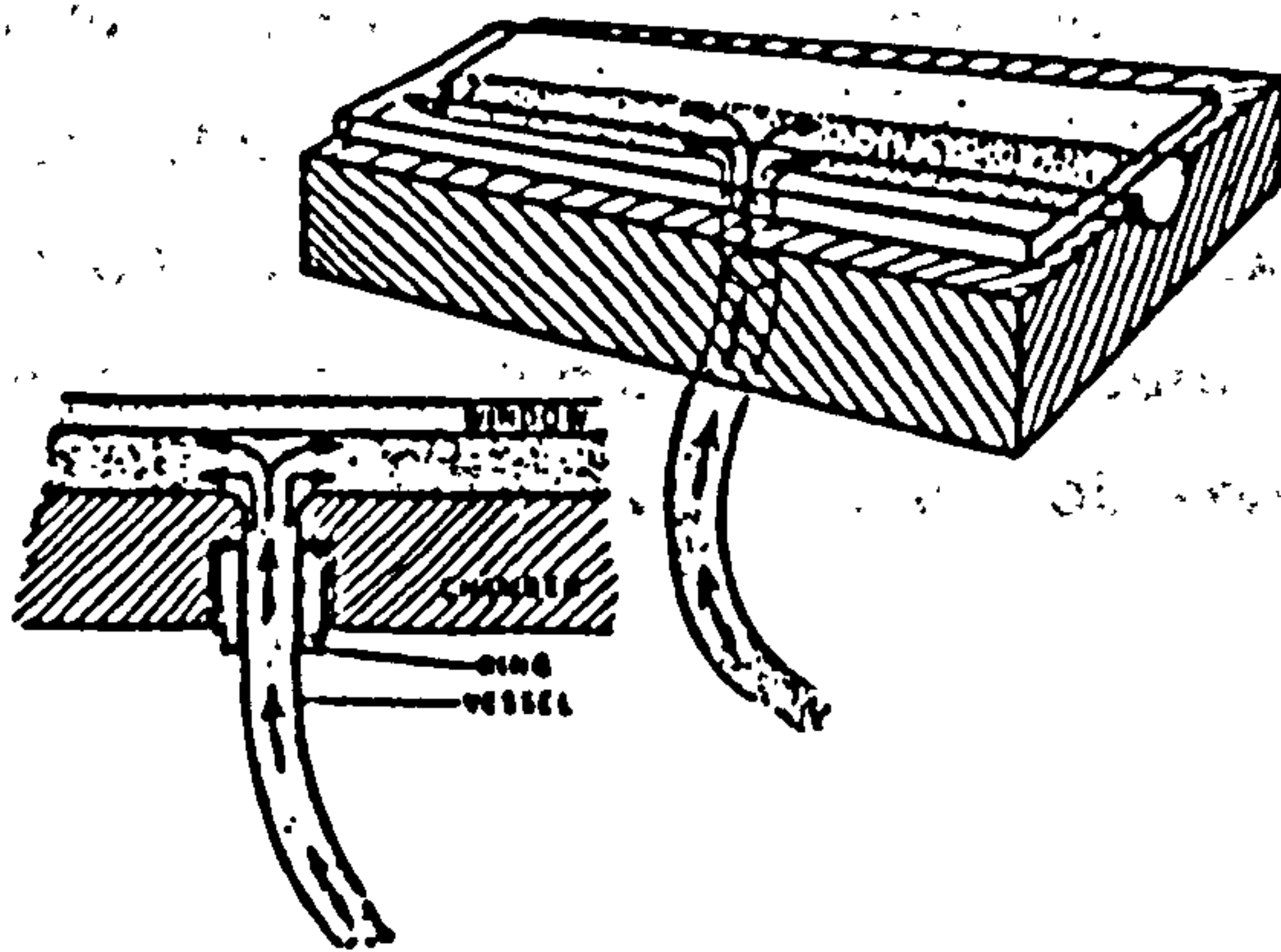


Fig. 10 Dutton's Blood Flow Chamber



The sequence of thrombotic events recorded by Dutton with this test chamber is in agreement with that observed by Petschek's group. Platelet deposition occurred before two minutes had elapsed, but no aggregates formed until after 3 or 4 minutes. These aggregates appeared at random positions on the window and led to the formation of many new isolated aggregates in their wakes. Thus thrombi, similar in appearance to the wedge thrombi reported by Petschek, were found to develop. Ultimately a fibrin mesh formed in the region between confluent wakes yielding a structure similar to a blood clot. Within these wedges the downstream thrombus contained more red corpuscles and fibrin with fewer platelets than did that upstream. The sites of initiation of these wedges were later seen to be connected with protrusions from the glass surface. General aggregation occurred in a region close to the line of flow division. The growth of this thrombus was dependent on the surface properties of the material.

Further investigation showed that the presence of heparin on a surface could inhibit the deposition of a fibrin mesh and that sudden changes in fluid velocity, brought about by manually pulling on the syringe, led to the production of emboli. The relevance of production of emboli by this means to emboli released from heart valves in pulsatile flow is not altogether clear, since pulsatile flow capable of dislodging such emboli will almost certainly prevent their deposition in the first place. Another set of experiments, drawing blood from the canine vein via a capillary tube at shear rates of  $104\text{s}^{-1}$ , in an attempt to provide a similar flow situation with the flow of fluid reversed, have shown that large aggregates form in the entrance region of the tube with the size of the aggregates decreasing downstream.

Friedman<sup>48</sup> et al observed heparinised canine blood flow, parallel to a surface, *ex vivo*. His test section comprised a rigid tube sliced along a chord with the material under test being inserted in the form of a flat plate across the open part of the tube (Fig. 11 ). Canine blood was drawn from the femoral artery or vein at various flowrates, a syringe pump being used for low flowrates, a roller pump at medium flowrates and the test section being connected as an arterio-venous shunt for high flowrates. At medium and high flowrates the blood was infused back into the experimental animal to avoid rapid depletion of blood volume. After exposure the plate was washed with saline, fixed in gluteraldehyde and observed by microscopy and microphotography.

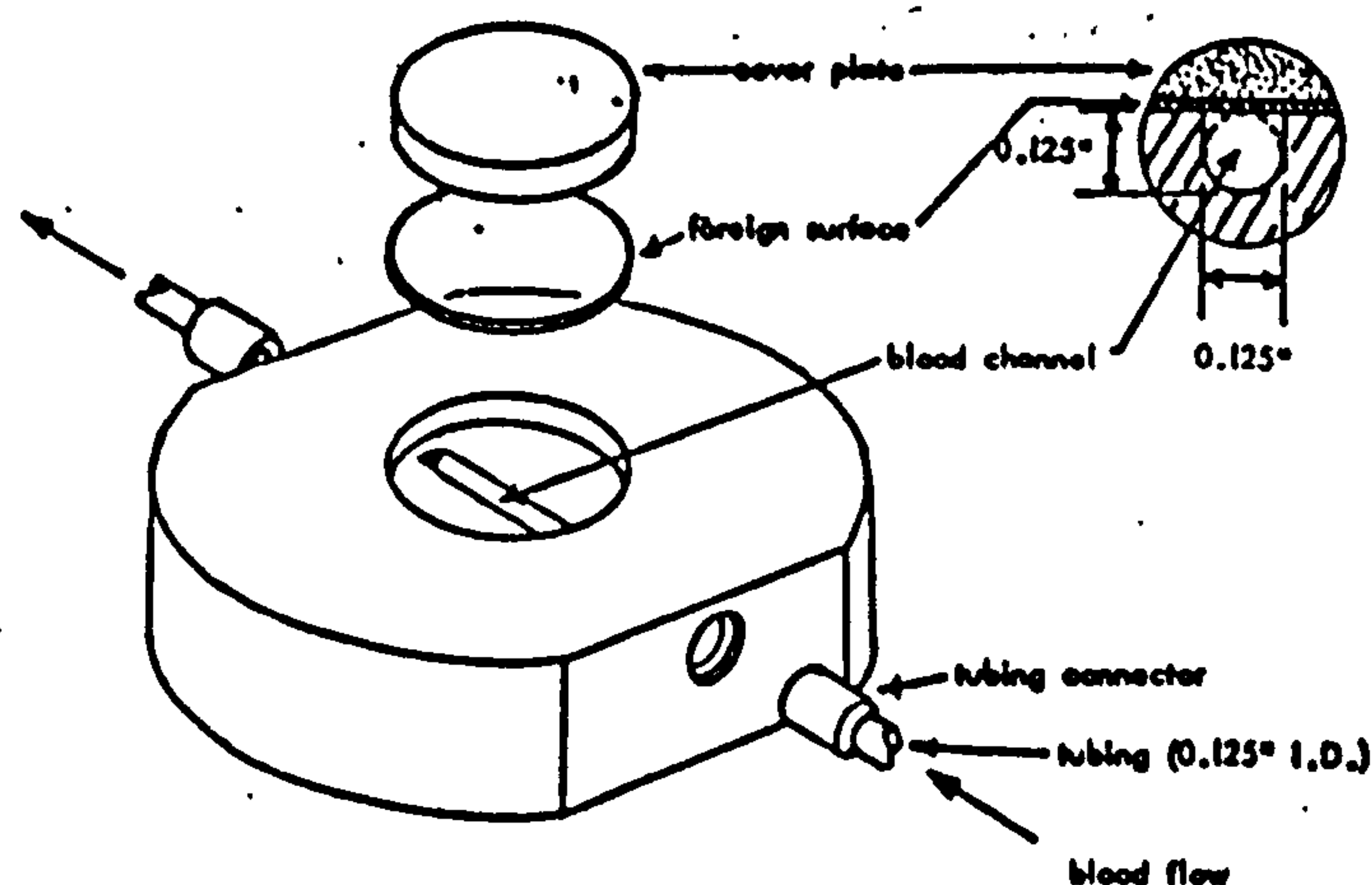


Fig. 11 Friedman's Blood Flow Chamber

The results suggest that the platelet density on the surface rises to a maximum value at a rate dependent on the flow, and will remain at this value indefinitely thereafter. The value reached appears to be independent of the flow for shear rates  $10-1000s^{-1}$  and of the surface material at high flowrates. Platelet aggregation was also measured on a variety of materials at high flowrates using non-heparinised blood. As with the heparinised blood the surface density of the platelets did not appear to be a function of the material. These experiments were limited to 10 minutes duration since at greater exposure times thrombus would form. Friedman et al therefore propose that the adhesion of platelets to surfaces in flowing blood is either diffusion controlled or that the surfaces are kinetically equivalent as far as adhesion is concerned.

### 3.2.3 *In Vitro*

Chandler<sup>96</sup> devised an *in vitro* test in which 1ml of venous blood is drawn into a flexible tube. The tube is then sealed by linking both ends to form a circle and this circular tube is rotated on a turntable inclined at  $23^\circ$  to the horizontal. A thrombus was observed to form at the anterior end of the column of blood. The front of the thrombus was composed of platelets and white cells whilst the tail was almost entirely red cells in a fibrin mesh.

Hladovec and Riha<sup>64</sup> carried out an *in vitro* test in which rat or human blood was pumped by a peristaltic pump from a cuvette, through a 50cm long, 3mm i.d. polyethylene tube with a glass pipe of equal diameter housing a nylon net at its outlet, and back into the cuvette (Fig. 12). The mesh size was 0.25mm for most experiments. Citrated whole blood, platelet rich plasma and platelet poor plasma were all used, being recalcified immediately prior to use in the experiment. The pressure drop across the mesh was recorded throughout the experiment with most visual observations only being made at the end.



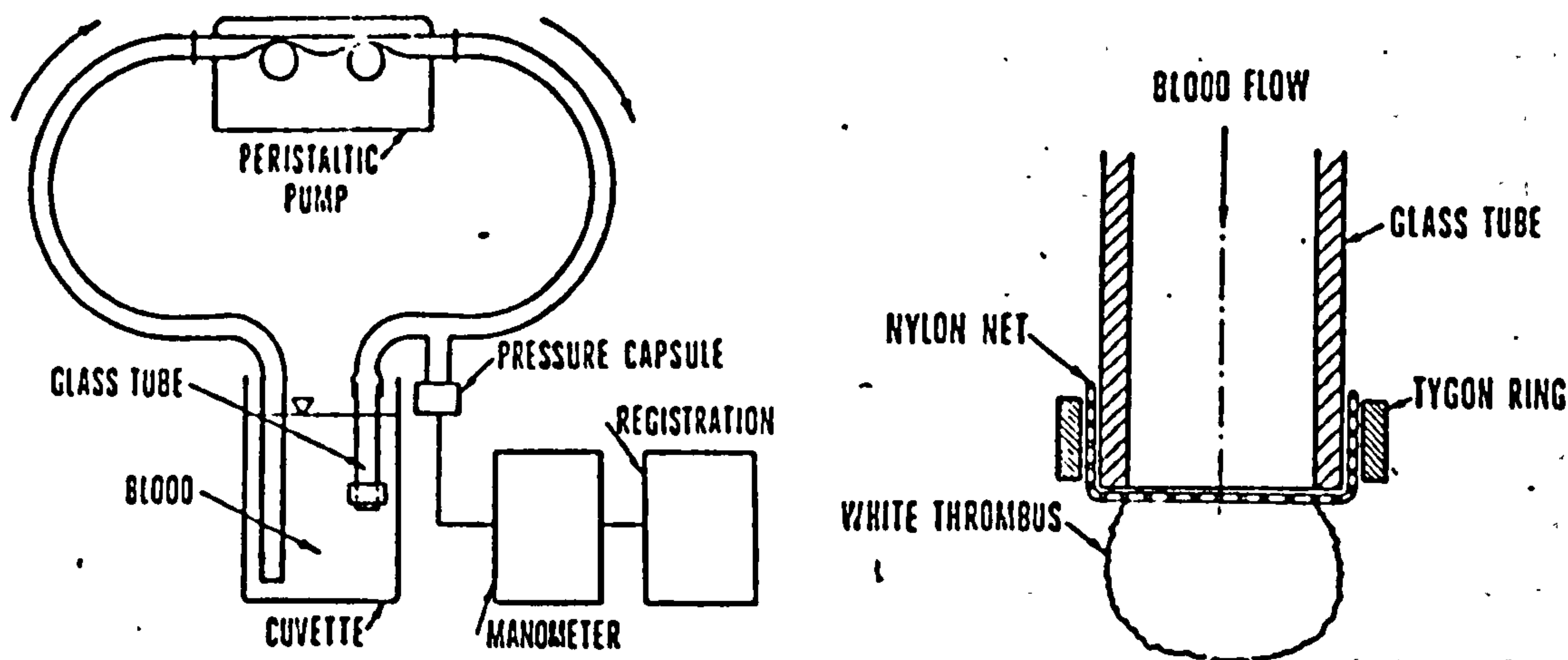


Fig. 12 Hladovec's apparatus and location of clot formed

The variation of pressure with time exhibited three distinct phases; an initial phase during which no change occurred, followed by a slow pressure rise during which some flocculation was observed and finally an abrupt pressure rise as a mass of white thrombus formed on the net. For whole blood at room temperature the initial phase lasted 27 seconds, the second phase 35 seconds and the final phase 10 seconds. Removal of the cellular components of the blood increased the length of the initial phase and decreased the duration of the final pressure rise. The duration of the intermediate phase was greatly influenced by the platelet concentration, being 30 seconds for platelet-rich plasma and 6 seconds for platelet-poor plasma. The thrombus formation times did not vary when the mesh size was increased from 0.25 to 0.4mm, but increasing the temperature to 37°C led to an increased clotting rate.

Of greatest interest, however, is the fact that the large growth of white thrombus was found to occur on the downstream face of the net, rather than the upstream or sieving side. Thus it would appear that a thrombus is actually developing preferentially in this region rather than forming elsewhere and being sifted out here. The structure of the thrombus was similar to that reported *in vivo* i.e. in the proximity of the net platelet aggregates abound with increasing amounts of fibrin and erythrocytes in the direction of flow. Hladovec accounts for the position of this thrombus by suggesting that turbulence induced by the net may first cause aggregation of platelets and then their gradual adhesion to the net. A more plausible explanation might be that of Lewis<sup>80</sup>, that particle deposition occurs in the small stagnation region downstream of each of the wires of the mesh, and that as deposition proceeds the size of this wake region will also increase allowing larger particles to adhere, sheltered from the shearing forces of the flow. In this case turbulence would only account for the initial activation of platelets and the release of clotting factors.



Seidel<sup>118</sup>, as an extension to his experiments in the canine heart, carried out an *in vitro* flow experiment using fresh canine blood. A free-floating ball was found to remain free of thrombus, presumably through a self-cleaning action in contact with the test chamber walls, whilst a sphere mounted on a sting collected a thick asymmetric thrombus on both its upstream and downstream faces.

Several other workers have observed the influence of controlled levels of shear, *in vitro*, on either whole blood or platelet-rich plasma. Glover<sup>128</sup> et al, using platelet-rich plasma in a concentric cylinder viscometer, observed that the initiation time shortened, the initial polymerisation rate increased and the mechanical strength of the clot decreased as the shear rate during formation increased. Dewitz<sup>129</sup> et al, also using a rotational viscometer, with shear rates in the range  $0-450\text{s}^{-1}$  and exposure times of up to 10 minutes, noticed that the mean size of the aggregates decreased with increasing shear. Turitto and Leonard<sup>130</sup> observed the deposition of thrombus on a spinning disc inside what was essentially an arterio-venous shunt. Initial platelet adhesion was slow but once platelet aggregation occurred the thrombus grew rapidly.

Goldsmith and Karino<sup>131</sup> have observed the behaviour of whole blood at a sudden expansion in a capillary tube. Platelet aggregates were observed when the Reynolds number, based on the downstream flow, was between 3 and 15. It was suggested that, at lower Reynolds numbers the shear stresses would be too low to cause sufficient platelet collisions for aggregation and that, at higher Reynolds numbers, the shear stresses would be large enough to break down the aggregates formed. When platelets adhered to the capillary tube wall they did so preferentially just upstream of the point of flow reattachment at the downstream end of the vortex. Goldsmith<sup>132</sup> has also observed that red cells will migrate into the vortex downstream of a spherical projection from the wall of the pipe.

### **3.3 Flow Measurement in Known Thrombogenic Environments**

A variety of experiments have been conducted to analyse flow properties in regions where blood is known to clot. Most of these investigations have centred on flow in the vicinity of prosthetic valves, though several have accompanied clotting experiments reported in the previous section. A range of techniques has been used, including flow visualisation, hot film anemometry and laser doppler anemometry to determine the structure of the flow, and electrochemical and holographic methods to determine mass transfer characteristics of the flow. In the following discussion each technique will be reviewed, together with the type of information obtained (with particular reference to findings related to blood flow). In all of these studies various

types of mock circulation apparatus have been used. These will not be discussed in detail: Further information is given in the references cited.

Any flow study will normally begin with direct visualisation of the gross structure of the flow, so that the use of time-consuming, more refined techniques can be limited to appropriate areas of interest. The simplest form of flow visualisation is that of injecting dye, such as naphthalene black<sup>133</sup>, into a flow of clear fluid. The movement of the dye can then be observed and photographed, yielding information on the macroscopic behaviour of the flow. Refinements can be made, for example, by using a dye that reacts with the clear fluid to become colourless after a known time interval (Useful if backmixing tends to obscure the behaviour of flow into a region), or by suspending reflective particles, such as Pearl Essence<sup>134</sup>, Gold particles<sup>32</sup>, plastic spheres<sup>135</sup> or even hydrogen bubbles<sup>136</sup>, in the flow and using slit lighting to observe the motion of these particles in one plane of a three dimensional flow system. If residence times are being sought, a clock reaction such as that used by Danckwerts<sup>137</sup>, can yield more information than dye injection studies.

Direct observation of the movements of valves in pulsatile flow can also yield interesting information. For instance the silastic ball of the Starr-Edwards valve was found to bounce in the base of the cage whereas the stellite ball did not<sup>138</sup>.

These studies, however, are not suited to measuring local fluid velocities and turbulence intensities, especially if the flow is of a pulsatile nature. For this reason, research into shear stress and turbulence intensities has relied upon more sophisticated techniques. Regions of stasis and flow impingement on surfaces can, however, be ascertained from such flow visualisation, but since most flow visualisation studies have been used for identifying regions of interest for more detailed study, the results of such studies will be included along with the more detailed flow analysis to avoid unnecessary duplication of text.

Hot wire/film anemometry was the first flow measurement technique to offer both the spatial and temporal resolution necessary for turbulent or oscillatory flow studies. Hot wire and hot film anemometry involve the insertion of a small heated probe into the flow. Since the rate of heat transfer depends on the local fluid velocity, the cooling rate can be used to measure this velocity. The probe can be operated in one of two modes. A constant current can be passed through the probe, the resulting temperature being measured by means of the probe's resistance or, more commonly, the probe can be maintained at a constant temperature with the heat supply rate being measured.



Hot film and hot wire anemometry can yield both mean velocity and turbulence measurements as their frequency response is normally good. Since the relationship between heat transfer rates and local fluid velocity is an empirical one, dependent on the extent of fouling of the probe, it is necessary to calibrate the system regularly. This involves inserting the probe into a number of flows of known velocity, since the response of the probe is non-linear – a time consuming process! The nature of this relation between heat transfer rate and fluid velocity will also depend on the direction of the fluid flow relative to the probe. Thus in extreme cases, such as reverse flow, the data recorded from such a system will be meaningless, and even changes of relatively small angles in flow direction will lead to ambiguity in the measured signal. Improvements can be made by the use of two probes perpendicular to one another since their signals, once correlated, will allow the measurement of two components of the velocity. Reynolds stresses can also be obtained from such correlations. In the flow studies which follow, only hot film anemometry has been used. Hot wire anemometry, whilst allowing for greater spatial resolution, is unsuitable for conductive or dirty flows and is thus recommended only for filtered gas flows. Unless stated, the fluid used in each experiment is an aqueous glycerol solution and the experiments have been conducted in a model aorta.

Nerem<sup>139</sup> et al have used hot film anemometry to measure flow velocities and turbulence power spectra in the canine aorta. Their results verify the presence of a vortex in the sinuses during systole that appears to assist closure of the valve, and suggest that the critical Reynolds number for the onset of turbulence may be proportional to the frequency parameter,  $\alpha$ .

Figliola<sup>71,72,140</sup> has used hot film anemometry with a dual cylindrical X-array probe in the vicinity of the Kay-Shiley, Bjork-Shiley and Starr-Edwards valves to measure turbulence intensities and Reynolds Stresses downstream of these valves in steady flow. The maximum shear stress recorded with the Kay-Shiley valve was 816 dynes/cm<sup>2</sup>, 0.9 diameters downstream of the disc (this being the closest position possible for the probe in relation to the valve). For the Bjork-Shiley and Starr-Edwards valves the values were 545 and 601 dynes/cm<sup>2</sup> respectively, again occurring at approximately one diameter downstream of the sewing ring. Turbulence intensities of between 40 and 50% were recorded with all three valves.

Hwang<sup>141</sup> has analysed the same turbulence characteristics downstream of a natural valve, this time in a 5l/min, 72 beats/min pulsatile flow. Longitudinal turbulence intensities of 25% and lateral values of 20% were measured. The maximum Reynolds stress recorded was 150 dynes/cm<sup>2</sup> and this occurred during diastole. Due to directional sensitivities of the hot-film probes, more than half

of the data was discarded, flow visualisation being used to assess the local flow direction and hence the reliability of the measured data.

Tillmann<sup>142,143</sup> mounted film probes in the sewing ring of both the Bjork-Shiley and Lillehei-Kaster valves in order to measure wall shear stresses in this region. The nature of these wall-mounted probes allowed for cooling rates being the same in both forward and reverse flows. With the Bjork-Shiley valve, at physiological pulsatile frequencies, a maximum wall shear stress of 180dynes/cm<sup>2</sup> occurred in the major orifice during systole, whilst during diastole 300dynes/cm<sup>2</sup> was recorded in the minor orifice, presumably as a result of regurgitation. In the pivoting axis region of the valve sewing ring, wall shear stresses of over 4000dynes/cm<sup>2</sup> were measured during diastole (200dynes/cm<sup>2</sup> during systole). With the Lillehei-Kaster valve the highest shear stresses are about 450dynes/cm<sup>2</sup> in the region of the pivots, occurring during systole.

Cassanova<sup>144</sup> has measured energy spectra in the region of a smooth-edged orifice by means of a conical probe in water. Distinct differences were observed between spectra obtained in steady flow at an orifice Reynolds number of 1270 and those obtained in pulsatile flow of frequency 0.2 Hz at the same mean Reynolds number. In steady flow a peak in the energy spectrum was observed at a frequency of about 7Hz at a point on the centreline, two diameters downstream of the orifice. This is believed to correspond to the shedding of vortex rings, and the absence of a marked peak in pulsatile flow would therefore suggest that vortex shedding, at least of constant frequency, does not occur to any great extent.

Laser doppler anemometry offers a number of advantages over hot film anemometry. The measurement technique relies upon doppler shifting of the frequency of laser light by moving particles in the fluid. Since the doppler shift is directly proportional to the particle's velocity in the direction of the light beam, no calibration is necessary. The technique is also non-invasive since only light enters and leaves the fluid.

The most common experimental arrangement is to split the laser beam in two, focussing these beams to cross over at the point of interest in the flow. Within this crossover region the monochromatic light beams interfere causing a pattern of light and dark fringes to form. If the cross-over region is at the beam waist of the Gaussian laser light beams these fringes will be parallel and any particle passing through these fringes will scatter light, the intensity of which will oscillate at a frequency (f) dictated by the fringe spacing and the velocity of the particle. The velocity of the particle will be given by

$$V_p = \lambda.f / (2.\sin(\theta/2)),$$

where  $\lambda$  is the wavelength of the laser light and  $\theta$  is the angle of incidence



between the two laser beams. This description constitutes the Fringe Model<sup>145</sup>. The same result can be achieved by calculating the vector difference of the light scattered from the two incident rays. If the particle in question is small enough so that its inertia is negligible then the particle will be travelling at the same rate as the fluid and the fluid velocity will therefore be known.

Since the technique relies on undistorted beams of laser light, the fluid medium and test section walls must be transparent. A further problem arises when using flow chambers of curved geometry; that of refractive distortion of the beams at the solid-fluid and solid-air interfaces. Distortion on the internal surface is generally less serious than at the solid-air interface and can be removed by using a fluid with a similar refractive index to that of the vessel wall. Distortion due to the external surface can be eliminated by either drilling out the test section from a solid rectangular block of material or by immersing the test section in a square-faced transparent bath containing a liquid of similar refractive index to that of the test section.

The frequency response of laser doppler anemometry is of the order of  $1\text{ms}$ <sup>146</sup> and the linear resolution can be as low as  $20\text{--}100\text{ }\mu\text{m}$ , depending on the quality and geometry of the optical system. Unlike hot film anemometry, directional ambiguity can be eliminated by shifting the frequency of one of the cross-over beams, thus causing the fringes to move in the direction of flow. Phase shifting is often used to accomplish this: This causes the fringes to move in a cyclical manner over a distance of one fringe spacing, travelling at constant velocity in the forward direction and 'flying back' almost instantaneously to the initial position at the end of its travel. Laser doppler anemometry is often chosen as a tool for measuring turbulent or oscillating flows due to this high resolution and directional sensitivity. However, a problem known as spectral broadening can lead to distortion of such results. In measuring velocities it is normal to take the average of signals received over a finite time period, and to attribute this average velocity to the fluid at the centre of the cross-over region. This volume is finite in size and so a scatter of frequencies may be obtained even when the flow is both steady and laminar. This scatter has three sources:

i) Velocity gradients in the flow<sup>147</sup>.

Since the probe length is finite, both fast and slow moving particles will be detected in a region of high velocity gradient. A further distortion occurs in 'burst' counting processors in that if the light-scattering particles are evenly dispersed, more will cross the probe volume per unit time in rapidly-moving fluid than in slow-moving fluid, weighting the velocity average towards a high

value. In photon correlation techniques, giving a time-averaged rather than a particle-averaged signal, this weighting should be less noticeable as faster particles spend proportionately less time in traversing the probe volume. The average particle-time signal should only depend on the width of, and light intensity in, the control volume at the point of crossing. Such processing techniques favour the velocity at the centre of the cross-over region where the passage width and the light intensity are greatest.

ii) Fringe gradients within the measuring volume.

Since the laser beams are Gaussian in nature the radius of the beams varies as

$$r = r_0 (1 + (\lambda z / \pi r_0^2)^2)^{1/2}$$

and the radius of curvature of the wavefront is given by

$$R(z) = z (1 + (\pi r_0^2 / \lambda z)^2)$$

(where  $r_0$  is the radius of the beam waist and  $z$  is the wavenumber).

If the optical system is misaligned so that the beams cross at a point other than the beam waist (where  $z=0$ ), the fringes will no longer be parallel, being formed from the interference of spherical rather than plane wavefronts. This will result in a range of frequency signals for particles of constant velocity passing through different points in the fringe volume<sup>148</sup>.

iii) Temporal coherence<sup>145</sup>.

If several particles of identical velocity are present within the sampling volume concurrently the interference of the light intensity signals of differing phase may lead to an apparent spectrum of frequencies being measured.

Another form of spectral broadening arises from the fact that 'monochromatic' laser light is actually composed of a narrow band of light frequencies. This causes variation in the fringe spacing such that the maximum number of discrete fringes that can be produced is  $\lambda/\Delta\lambda$ . The actual number of fringes may be less than this number. The error in determining the doppler shift  $\Delta f_D/f_D$  is at least  $\Delta\lambda/\lambda$  and is thus potentially the reciprocal of the number of cycles in the doppler train if the fringe number is limited by coherence considerations<sup>145</sup>.

This spectral broadening leads to ambiguity, both in the mean velocity and in the turbulence intensity, being more serious in the latter case. If turbulence intensities are desired it is thus necessary to reduce the size of the cross-over



region to the smallest possible, whilst maintaining a reasonable number of fringes ( $>15$ ) within the probe volume, i.e. to use as wide an angle as is practicable between the incident beams. Even then, in regions of high velocity gradient the information received may be misleading. Burst counter verification techniques have improved the quality of data received by ignoring individual doppler bursts of very short duration, such as occur when particles pass through the edge of the region or when particles change velocity within the probe volume. Such data discarding mechanisms may, however, cause the instrument to ignore information of value, particularly if the mean free path of particles following turbulence eddies is not large compared with the fringe spacing. Laser doppler anemometry is thus a difficult technique for obtaining unambiguous information in turbulent flows.

Chandran<sup>149,150</sup> has used one component LDA in steady flows of both saline solution and an aqueous glycerol solution (having the same kinematic viscosity as blood) in the vicinity of a Hall-Kaster, Bjork-Shiley and Ionescu-Shiley prosthesis. His results show that differences in peak velocities are present, especially with the Bjork-Shiley valve, when comparing the two solutions.

Figliola<sup>71</sup> measured the magnitude of the longitudinal component of velocity in a model of the aortic root using single component LDA. He studied the behaviour of steady flows of an aqueous glycerol solution in the vicinity of Kay-Shiley, Bjork-Shiley and two Starr-Edwards valves. Wall shear stresses in a range high enough to damage red blood cells and platelets were predicted from the mean velocities at a point just downstream of the reattachment of recirculating flow within the sinuses. These shear stresses were greater than  $2500\text{dynes/cm}^2$  for the Kay-Shiley prosthesis and just less than  $1000\text{dynes/cm}^2$  for the other valves. Measurements of wall shear on the surface of the ball in the Starr-Edwards valve revealed stresses as high as  $2840\text{dynes/cm}^2$ . These stress measurements are substantially higher than those recorded using hot film anemometry. It is possible that a significant proportion of these stresses arise from the measurement technique for the reasons outlined above.

Yoganathan<sup>73,151-153</sup> has also studied steady flow in a model of the aortic root using a Pluracol solution as a blood analogue. Investigation of the flow round the Bjork-Shiley valve revealed a maximum wall shear stress, predicted from mean velocities, of  $700\text{dynes/cm}^2$  in the major orifice<sup>151</sup>, in agreement with Figliola's findings for the same valve. In flow studies around the Smeloff-Cutter, Cooley-Cutter and caged-ball and caged-disc Starr-Edwards valves, wall shear stresses of just below  $1000\text{dynes/cm}^2$  were discovered for all the valves, being exceeded only by the Starr-Edwards 6520-1M caged-disc valve<sup>152</sup>. It was proposed that these stresses might lead to damage of the endothelium<sup>153</sup>. Turbulence intensities of between 25 and 50% were measured.



Hanle<sup>154</sup>, also using single component LDA, attempted to predict Reynolds stresses by measuring the three components of velocity and their r.m.s. fluctuations at the same point in the flow. Since the correlation coefficient between the velocities lies between 0 and 1, he proceeded to assume a constant value and to calculate turbulent shear stresses on this basis. He also introduced pulsatile flow into his experiments, collecting data during a small fraction of the pulsatile cycle and averaging over a large number of cycles. From Hanle's study of the Ionescu-Shiley, Bjork-Shiley, Smeloff and St. Jude valves in an aortic root, the St. Jude valve emerged as having the lowest pressure drop in forward flow, the lowest turbulence intensities and shear stress, and the second lowest leakage in reverse flow.

Bruss<sup>155</sup> et al have measured axial velocity profiles in the maximum diameter region of the aortic root, downstream of the Bjork-Shiley standard, Bjork-Shiley Concavo-Convex, Hall-Kaster and St. Jude valves, over a range of phases of the pulsatile cycle. Again the St. Jude prosthesis looked promising in relation to the distribution of forward flow through the valves. A problem did emerge, however, that has also occurred clinically; that of blockage of the hinges by large particles ( $>200\mu\text{m}$  diameter).

Woo<sup>156</sup> has studied both steady and pulsatile flow downstream of a trileaflet valve using single component LDA. He discovered that the flow through such a valve had a tendency to take the form of a jet. Tiederman<sup>69</sup> and Walburn<sup>157</sup> have since measured Reynolds stresses by an improved technique, that of two component LDA. Since both components of the flow are measured simultaneously, Reynolds stresses can be determined directly without having to make assumptions regarding correlation coefficients. Using a thin-walled transparent cylinder to represent the aorta, Tiederman measured total shear stresses (i.e. viscous and turbulent stresses) one and two diameters downstream of a tri-leaflet valve and a Lillehei-Kaster tilting disc valve. Maximum total shear stresses of 2820 and 2070 dynes/cm<sup>2</sup> were found one diameter downstream of the trileaflet and Lillehei-Kaster valves respectively for data recorded at peak systole. Walburn measured both Reynolds normal and Reynolds shear stresses over the whole pulsatile cycle for a Hancock porcine valve. Maximum shear stresses of 2500 dynes/cm<sup>2</sup> were measured, though the average over the whole cycle was only 38 dynes/cm<sup>2</sup>. The corresponding normal stresses were 6800 and 380 dynes/cm<sup>2</sup> respectively.

Forstrum<sup>158</sup> has used laser doppler anemometry to measure the velocity and distribution of ghost red cells flowing between parallel plates, a distance of 416 - 2300  $\mu\text{m}$  apart. A cell-depleted region was observed extending up to 15  $\mu\text{m}$  from the wall. Fluctuation velocities of the ghost cells of the order of 15% were measured. Enhanced platelet transport caused by this movement of



cells was proposed as a mechanism to explain higher than expected diffusion rates for platelets.

Since platelet transport to a surface is thought to be important in determining the deposition rate, a few experimenters have estimated local mass transfer coefficients in regions of flow where thrombosis is known to occur. It was thought that a correlation might exist between regions of high mass transfer coefficient and favourable sites for thrombosis. Taylor<sup>70</sup> used a holographic technique to measure the recession of a swollen polymer film, due to evaporation of solvent, in a test chamber identical to that of Petschek. He found a constant mass transfer coefficient within the impingement zone of the jet, showing that the radius of the white cell circle in Petschek's experiments is not limited by local mass transfer considerations.

Galanga<sup>159,160</sup> has used an electrochemical technique, where the reaction at the electrode is diffusion-controlled, to measure local mass transfer coefficients. The electrode length is kept short to prevent the build-up of a concentration boundary layer. Thus the current measured is proportional to the rate at which ions are carried to the electrode by the fluid. Ferrocyanide ions in 2M aqueous NaOH is used as an electrolyte. For the Starr-Edwards valve in an aortic test section two regions of high mass transfer were located; one just upstream of the sewing ring, the other on the wall at a point approximately in line with the minimum annular gap caused by the ball.

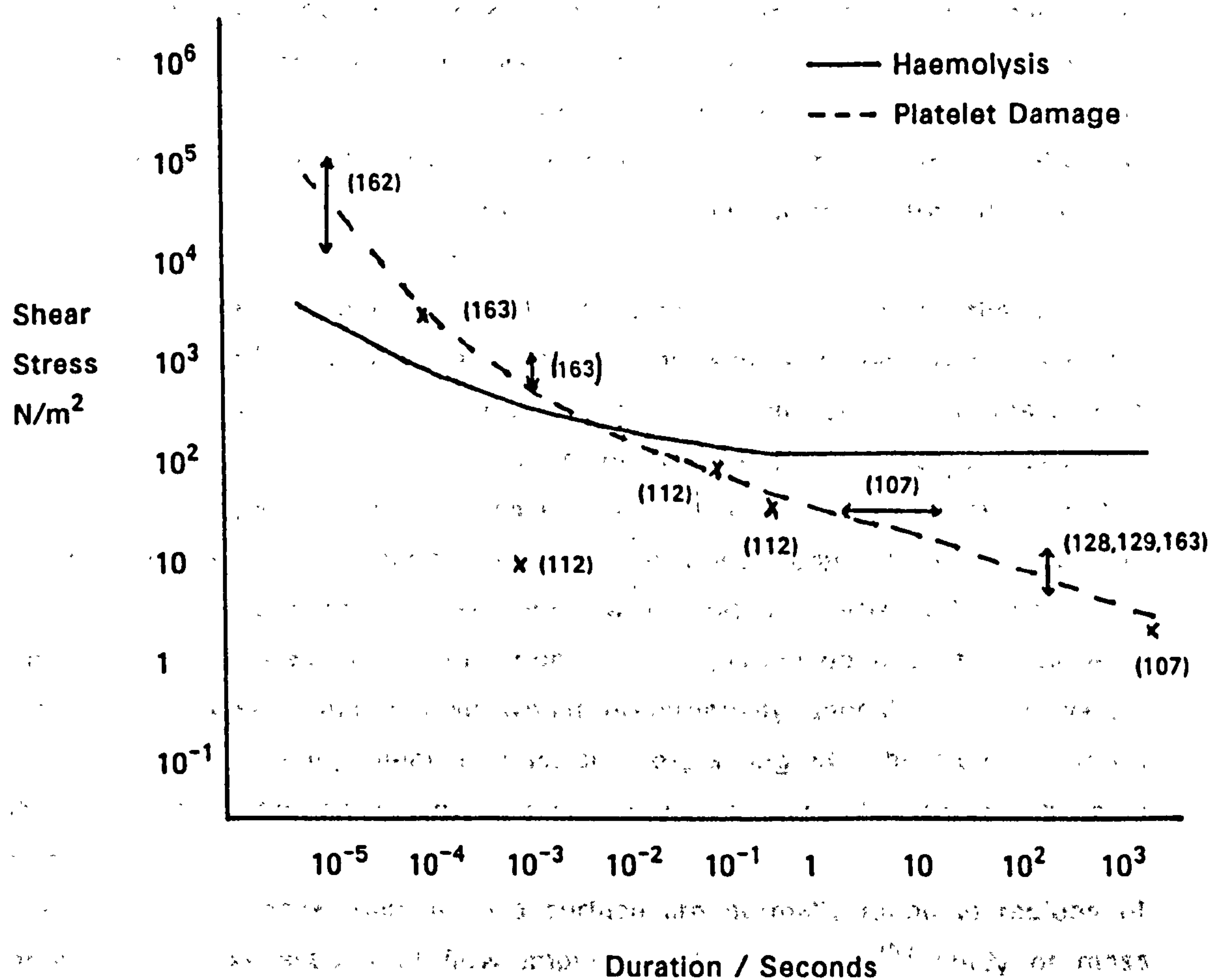
### **3.4 Summary of Effects of Flow Properties on Thrombosis**

Tying these experiments together, it can be seen that whilst elevated shear, such as occurs in turbulence or in high mean velocity gradients, may be associated with increased coagulation rates there is by no means a direct relationship between the two. Vorhauer<sup>121</sup> found that increased wake turbulence, downstream of bluff bodies, led to greater clot deposition, as did Smith<sup>123</sup>, Stein and Sabbah<sup>124</sup> in the wake of orifices, but that free stream turbulence further downstream led to less rather than more thrombus in the mounting ring. Petschek<sup>66</sup>, on the other hand, found that increased shear resulted in less deposition, so that, except at imperfections, the test surface remained thrombus-free in his stagnation point flow chamber experiment. Friedman<sup>48</sup>, in his work on platelet deposition found no difference in the quantity of platelet deposition in varying the surface shear rate from  $10 - 1000\text{s}^{-1}$ .

This confusion is further compounded by the observation in LDA studies that higher total shear stresses are recorded in the region of a trileaflet valve than around a tilting disc valve, whereas anticoagulation therapy, normally recommended for tilting disc valves, is not usually required for trileaflet valves:

Anticoagulation therapy is normally only administered where clinical practice has shown it to be necessary.

One possible explanation of the above inconsistencies is that whilst high shear may render platelets aggregable or cause release of coagulation-inducing chemicals, no coagulation will result unless the activated species enter a region adjacent to a solid surface with favourable flow characteristics for deposition to occur. Elevated shear stresses are known to cause damage to both blood and the endothelium. Fry<sup>111,161</sup> has shown that wall shear stresses above 400dynes/cm<sup>2</sup> will lead to endothelial damage and that cell erosion will occur above 950dynes/cm<sup>2</sup>. Damage to formed elements of the blood occurs when the shear stress exceeds a critical level, the value of which is dependent on the exposure time. A graph of this critical shear stress versus exposure time for haemolysis was drawn by Leverett<sup>105</sup> from collated data. This is redrawn in Figure 13 together with a similar relationship for platelet damage, based on the references cited<sup>107,112,128,129,162,163</sup>.



**Fig. 13** Curves of critical shear stress versus exposure time for haemolysis and platelet damage (Reference numbers in parentheses).



The shear stresses measured by hot film anemometry and LDA are sufficient to cause both red cell and platelet damage. Such damage is known to release clotting agents into the blood and may thus render the blood hypercoagulable. Endothelial damage has the added effect of providing a favourable site for deposition<sup>164</sup>. Shear is also known to denature factor X and may thus also serve to inhibit the action of the clotting reagents released<sup>114</sup>.

From Petschek's experiments it would appear that high surface shear might indeed inhibit platelet deposition, but if the damaged particles or released chemicals were to enter a region of stasis next to a surface, deposition might occur. In the case of trileaflet valves, the differences between the observed shear rates<sup>69</sup> and expected thrombogenicity may be due to two factors: The high shear rate in the case of the trileaflet valve is associated with the formation of an axial fluid jet, thus any elements damaged by shear will be swept downstream rapidly from the valve without contact with a solid surface, and endothelial damage will be minimal due to the central flow of the jet. With the tilting disc valve the flow is directed at the wall giving surface contact and possibly endothelial damage. Leakage through a valve when closed, leading to high wall shear stresses, such as those measured by Tillmann<sup>142</sup> during diastole, is one possible source of damage where the released agents may be harmful, entering recirculating fluid in the heart chamber. Paradoxically the sealing face of the valve housing should remain thrombus free due to the cleaning action of this high shear stress.

Areas where flow impinges on a surface may or may not be the sites of thrombosis. Gott<sup>62</sup>, for instance, found with his square-edged orifices that the point of flow impingement on the upstream face of the orifice was the site of initiation of thrombus growth. Fairing of this upstream face removed this impingement zone and in some cases inhibited clotting. Petschek<sup>66</sup>, on the other hand, using a stagnation point flow chamber, only observed thrombus within the impingement zone at very low flowrates. Vorhauer<sup>121</sup>, too found that clot did not generally form around the stagnation point on the upstream face of the objects, but instead would preferentially deposit in their wakes. Thus whilst flow impingement may assist in explaining why thrombus forms on the upstream of the sewing ring in clinical practice, it is unlikely to be the only factor involved.

High rates of mass transfer to a surface are normally found in regions of high shear or in regions of flow impingement. Galanga's<sup>159</sup> study of mass transfer in a mock aortic flow chamber revealed that high mass transfer rates were observed upstream of the orifice where thrombus is known to occur. However, his study also revealed high rates of transfer to the wall where the ball most obstructs the flow. This is not normally a site of thrombosis, unless

endothelial damage is present. Taylor<sup>70</sup> has also shown, using a stagnation point flow chamber, that the local mass transfer coefficient remains constant over the whole of the impingement zone of the jet, falling with radial distance outside this region. However, neither the radius of the white cell circle, nor that of the symmetrical thrombi formed in low flow, appear to be determined by the rate of mass transfer. The rate of growth of wedge thrombi may, however, depend on the rate of mass transfer. Petschek<sup>104</sup> points out that the growth of these thrombi, from their point of inception at a surface imperfection, occurs most rapidly downstream of the thrombus head and explains this in terms of flow-assisted diffusion of clot-inducing chemicals (released from adhering aggregates of platelets) into this region; growth upstream being inhibited by a reduction in upstream diffusion due to the flow. Whilst mass transfer is essential to thrombosis, being the process by which aggregating elements reach the surface and whilst it may be the rate-limiting factor for thrombus growth, it does not appear to be responsible for the initiation site of thrombus deposition, a surface imperfection seemingly being required for Petschek's wedge shaped thrombi.

A further effect, not accounted for in Galanga's and Taylor's experiments, is that of enhanced platelet diffusion brought about by red cell tumbling<sup>158</sup>. This may in some instances be the predominant mechanism of diffusion, rendering conventional mass transfer measurement techniques inaccurate for estimating mass transfer rates in blood flows.

Stasis is thought by many to determine the sites where thrombus will grow. Blackshear<sup>63</sup>, with regard to the Gott ring experiment, postulates that thrombus will occur if the recirculating vortex downstream of the orifice extends beyond the the end of the mounting tube, thus allowing chemicals released from the damaged intima to be drawn into the entrapped fluid. This, as mentioned earlier, is doubtful if, as is reported, the clot generally initiates on the upstream face of the orifice. Vorhauer<sup>121</sup>, too, implicates stasis as a factor since clot deposition around his test bodies generally occurs in their wakes. Leonard<sup>125</sup> also observed thrombus to form in a region of stasis, that of a circumferential groove in a 'laminar flow' pipe. Clot formed primarily in the corners of the groove and was not observed in the rest of the pipe. Stein and Sabbah<sup>124</sup> on the other hand found thrombosis downstream of a tapered orifice, where in dye injection experiments prolonged stasis was not observed. The position of thrombus in Hladovec's<sup>64</sup> net experiment suggests that the wake of the net is important. Here no large scale stasis is present and viscous and turbulent shear may be expected to be the dominating flow properties. However stasis on a small scale will almost certainly occur immediately behind the struts of the mesh, and whilst little is known regarding its influence on deposition, it



may prove to be the cause of thrombus deposition in this region.

Goldsmith and Karino<sup>131</sup>, in an elaborate visualisation experiment of the movement of red blood cells and platelets downstream of a flow expansion, have observed that cellular components and platelet aggregates tend to become trapped in such recirculating regions. Their experiments were carried out in steady flow, so doubt must be cast on whether such entrapment will occur in a vortex formed in pulsatile flow, regardless of whether the vortex itself remains trapped.

Tillmann<sup>142</sup>, Figliola<sup>72</sup> and Yoganathan<sup>73</sup> in their flow studies have all linked regions of stasis, usually observed in steady flow *in vitro*, with observed sites of thrombus formation *in vivo*. It remains to be seen whether such sites of stasis occur in physiological flow regimes.

One aspect of stasis that is almost certain to play a part in deposition is that of prolonged contact of aggregating species with foreign materials in the blood. An investigation of surface contact times for blood in pulsatile flow might prove interesting.

From the above discussion it is apparent that whilst elevated shear stresses, high mass transfer rates and stasis all influence thrombosis, the relative importance of each of these effects is by no means certain.

## Chapter 4. Milk as a Blood Analogue Fluid for Thrombogenicity Assessment

### 4.1 Requirements of an analogue fluid

Most of the experiments performed on heart valves to assess their thrombogenic potential, or to ascertain hydrodynamic correlates of thrombosis, have been limited to the observation of pressure drops, shear stresses, flow profiles and mass transfer coefficients in the hope that one factor, or possibly a simple combination of factors, may be the cause of thrombosis around prosthetic heart valves. As can be seen in the previous chapter, much is already known regarding some types of flow that may lead to thrombosis, but too many anomalies exist to allow firm deductions to be made from this data.

Current practice for assessment of thrombogenicity involves implantation of the device in a living animal (normally a dog or a calf for prosthetic heart valves). As mentioned in the Introduction this is unsatisfactory for comparative tests due to inter- and intra- species differences in blood clotting behaviour, not to mention objections of high cost and lack of consistency in surgical technique between test centres. In order to be able to test directly for thrombosis it would be advantageous to have an *in vitro* test in which the clotting of blood could be simulated in a controlled manner. A brief mention will be made here of the criteria for dynamic similarity in pulsatile flow followed by a discussion of the requirements of a suitable analogue fluid.

Mueller<sup>138</sup> and Martin<sup>165</sup>, amongst others, have emphasised the need for standardisation of prosthetic valve testing, principally in relation to the evaluation of pressure drops, shear stresses and flow profiles, if useful comparisons of performance are to be achieved. Schultz<sup>74</sup> has suggested that the Reynolds number,  $Re$ , and the frequency parameter,  $\alpha$ , are the standard ratios for ensuring dynamic similarity in pulsatile flow. Nerem<sup>139</sup> has shown that the onset of turbulence in pulsatile flow does indeed depend on both  $Re$  and  $\alpha$ , suggesting a relationship of the form  $Re_c = C * \alpha$  for the critical value of  $Re$ , above which turbulence is expected to occur. If standard-sized valves are to be tested, the flow chamber must be of the same general shape and size as the physiological situation. Thus if  $Re$  and  $\alpha$  are to be the same as those encountered *in vivo*, the pulsatile frequency, kinematic viscosity and volumetric flowrate must all be scaled by the same factor in relation to physiological values.

Blood is a difficult and hazardous fluid to use *in vitro*, requiring anticoagulants to prevent clotting during storage and being capable of spreading infection. Also, for *in vitro* testing of a standard-sized heart valve in



a single-pass system to prevent circulation of clotted material, a bulk flowrate of about 30 gallons/hour would be required to faithfully simulate physiological flow. A more readily available, safe alternative to blood has thus been sought.

Such a blood analogue must mimic a number of the properties of blood. In attempting to measure the location and deposition rates of coagulum the chosen analogue must be capable of depositing clot in identical locations and in similar relative amounts to those found with blood in similar flow regimes *in vivo*. This also leads to the requirement that the rheological behaviour of the formed clot must be comparable with that of thrombus, otherwise the growth characteristics and structure of the deposit will not vary in the same manner with flow variations as does thrombus. Linked with this is the necessity that the clot should be structurally stable, remaining bound to the surface on which it has formed until its size and shape have been determined. Naturally, if the analogue is to be an improvement over the use of blood *in vitro*, it must be safe to use and readily available at low cost.

The prospect of finding an analogue fluid for blood in studies of thrombogenicity at first sight would seem slim. The coagulation of blood involves a cascade of enzyme-catalysed reactions. There seems little hope of being able to model this aspect of the behaviour of blood. Consideration of the nature of thrombosis and of the prime requirement of the model, that of determining the location of, and providing quantitative discrimination between, clotting sites, suggests, however, some important simplifications. In blood it is known that many factors, such as damage to the endothelium<sup>11</sup>, red blood cells or platelets, or the presence of a foreign material, may trigger the cascade of reactions leading to coagulation. With the clinical use of prosthetic heart valves at least two of these factors are present: Endothelial damage is necessarily present where the sewing ring is stitched into the cardiac tissue, and foreign material is present in the valve itself. Thus it might be expected that the blood passing the valve in forward flow is already in a hypercoagulable state. If this is the case, then the important question concerning the behaviour of blood as it flows past the valve is whether fibrin, forming from fibrinogen by the action of thrombin, will deposit on the valve surface.

The analogue fluid in such a model need therefore only mimic the latter stages of blood coagulation; the conversion of fibrinogen to fibrin and the polymerisation of fibrin to form a mesh-like clot. A further assumption is implicit in the discussion so far; that the growth of platelet aggregates without the stability afforded by a fibrin mesh is unlikely to pose a major problem. This is almost certainly the case, since, due to the continual movements of the valve, such aggregates may be expected to become dislodged before they grow to a size that will impair valve function. (There is, however, some

evidence that the operation of the St. Jude valve may be impaired by the presence of 200 $\mu$ m particles in the flow<sup>155</sup>). These dislodged fragments are less likely to be as dangerous in the microcirculation as thromboemboli composed of aggregates bound in a fibrin mesh since they are easily fragmented.

To summarise, the necessary characteristics of a suitable blood analogue fluid for thrombogenicity assessment are as follows:

- i) It should simulate blood clotting to the extent that it clots in similar locations and forms deposits whose growth characteristics are similar to flowing blood. This implies either a kinetically similar clotting reaction to that of fibrin, if kinetic effects are dominant, or similar diffusive behaviour of clotting elements if the reaction is diffusion-limited. Observations presented later in this thesis (Chapter 7) suggest the former is the case.
- ii) The rheology of the formed clot should be similar to that of thrombus.
- iii) The clot should adhere to a solid surface, otherwise deposits will be swept away before measurements can be made.
- iv) The fluid should be safe to use.
- v) The fluid should be readily available at low cost.

## 4.2 Possible Analogue Solutions

A number of fluids undergo coagulation or aggregation reactions. Different aspects of coagulation have been studied in the following fluids by the research workers cited: CuO Sols<sup>166</sup>, FeOOH Sols<sup>167,168</sup>, Silver hydrosols<sup>169</sup>, Silver Bromide Sols<sup>170</sup>,  $TiO_2$  Sols<sup>171</sup>, Silica Dispersions<sup>172</sup>, Gold Sols<sup>173</sup>, Quartz Dispersions<sup>174</sup>, Noble metal Sols<sup>175</sup>, Galena dispersions<sup>176</sup>, Haematite Sols<sup>177</sup>, Nujol dispersions<sup>178</sup>, wax dispersions<sup>178</sup> and a range of polymer lattice dispersions<sup>179,180</sup>. Most of these studies, particularly those of Heller and Freundlich on CuO Sols, FeOOH Sols and Polystyrene lattices, have centred on testing the validity of Smoluchowski's theorem, relating aggregation to the collision frequency of particles, which would naturally be enhanced by the presence of a shear field. The results however have suggested that these particular coagulation reactions occur primarily at the vapour-liquid



interface<sup>168</sup>. For example, Freundlich<sup>166</sup>, using CuO and FeOOH sols, found a zero order coagulation reaction. The rate of aggregation appeared to vary as the square of the stirrer speed and the solution would not coagulate in the absence of an air/liquid interface<sup>167</sup>. The behaviour of these solutions on the addition of electrolyte would tend to suggest the stability of these colloidal solutions depends on the relative values of the attractive London Van der Waals forces and the repulsive electrostatic forces represented by the  $\zeta$ -potential (Theory of Lyophobic Colloids<sup>181</sup>). This would also explain why coagulation would proceed at an air/liquid interface, where the  $\zeta$ -potential is generally lower, in an otherwise stable colloidal solution. Colloidal aggregates relying on these forces for structural stability are unlikely to grow to significant size, if indeed they will bind to a solid surface. Inorganic colloids were therefore considered unsuitable as analogues for blood.

Tombs<sup>182</sup> has studied the gelation of globular proteins, attributing the formation of a gel to cross-linking of protein strands between globules. This bridging between particles gives a more rigid clot structure than is the case with inorganic colloids, suggesting that a protein solution might best simulate the latter stages of thrombosis. Several proteins such as gelatin, Myosin, Albumin, Fibrinogen<sup>183</sup> and Haemoglobin<sup>184</sup> are known to form coagulum gels under the action of heat. Others, such as Procollagen<sup>185</sup>, Ovalbumin<sup>186</sup> and Casein<sup>187</sup> (present in milk) undergo enzymic coagulation. Since blood is to be modelled as closely as possible, a solution is required that has similar coagulating characteristics. Therefore a solution that undergoes enzymic coagulation is chosen, making possible observations of the effects of production of the coagulating protein within the flow system. Milk has been chosen as such a coagulating protein due to reported similarities between its coagulation and that of blood, and because it is cheaper and more readily available than purified protein solutions.

#### 4.3 Milk as a Chemically Suitable Analogue

A number of investigators<sup>79-82</sup> have observed similarities between the clotting of milk and the latter stages of thrombosis. Macroscopically, both reactions exhibit two phases; an induction phase during which enzymic activity appears to dominate and a secondary coagulation phase during which a mesh-like clot is formed. Berridge<sup>81</sup>, for instance, has compared both the enzymic nature of the two reactions and the role of calcium ions in the secondary phases. Consideration will first be given to the current knowledge of the clotting of milk before returning to compare the two coagulations.

Milk is an aqueous solution containing both fat globules and protein micelles<sup>188-190</sup>. The micelles contain several proteins, the important ones

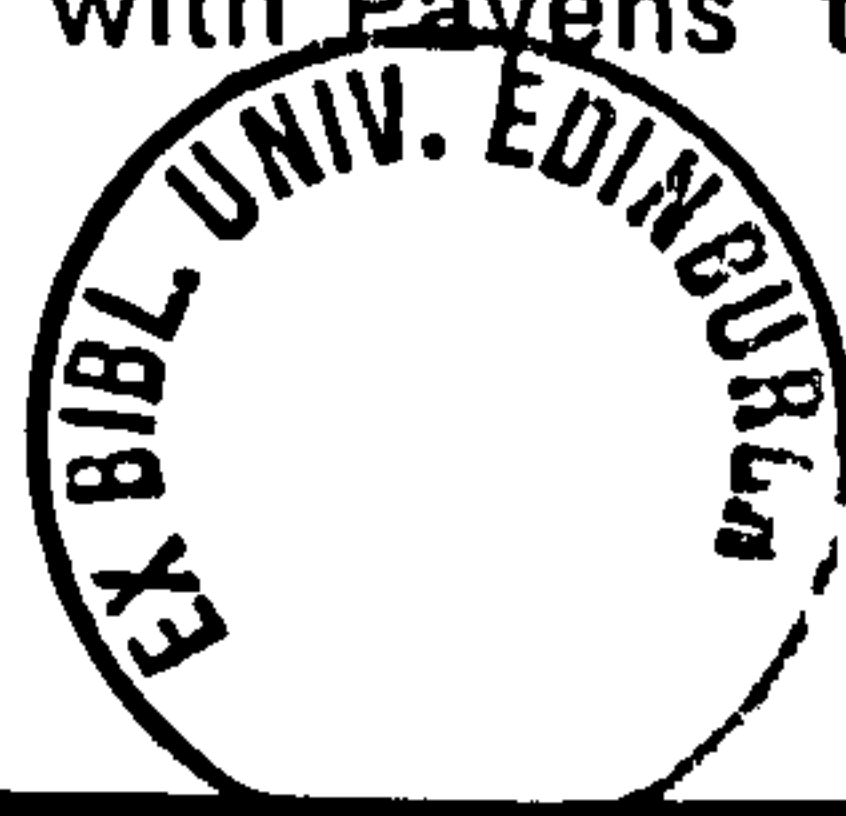
appearing to be  $\alpha_s$ -,  $\beta$ - and  $\kappa$ - caseins. All but  $\kappa$ -casein are hydrophobic.  $\kappa$ -casein, having a hydrophilic C-terminal section and a hydrophobic N-terminal, is thought to give stability to the protein micelles in milk, interacting with both water and the hydrophobic  $\alpha_s$ -casein<sup>82,191</sup>. For this reason  $\kappa$ -casein was first identified as the 'protective colloid'. This  $\kappa$ -casein is thought to be dispersed throughout the micelle with as much as 70% being contained in the surface layer. The primary phase of the milk clotting reaction is the cleavage of an N-P-N bond<sup>192</sup> in  $\kappa$ -casein by chymosin (Rennin) to yield an insoluble protein (para- $\kappa$ -casein) and a soluble glycopeptide<sup>193</sup>. This conversion of  $\kappa$ -casein to para- $\kappa$ -casein renders the protein hydrophobic so that the micelle becomes unstable in solution.

Payens<sup>194-196</sup> has proposed a theory to explain the apparent dual phases observed in milk (and blood) clotting. He assumes that the enzymic attack on the substrate is a zero order reaction and that the polymerisation of the monomer proceeds by means of binary collisions leading to combination, as proposed by Smoluchowski<sup>197</sup>, and is thus a second order reaction. Payens proposes that these reactions proceed concurrently and that observable effects of coagulation will vary as the weight-average molecular weight of the polymer formed. Using the Michaelis-Menten Equation for the action of enzyme on the substrate, with excess substrate present, the rate of production,  $V$ , of the monomer is zero order and proportional to the enzyme concentration,  $C_e$ . Solving the equation for the rate of change of molecule numbers:

$$d\Sigma P_i/dt = V - k_s(\Sigma P_i)^2$$

where  $P_i$  is the number of polymers containing  $i$  monomer molecules and  $k_s$  is the rate constant of polymerisation (assumed to be independent of the size of molecules reacting, as in Smoluchowski's early theory), a delay is observed before the weight-average molecular weight of the polymer begins to rise rapidly. This delay time is proportional to  $(k_s V/2)^{-1/2}$ . Thus if  $k_s$  is independent of  $C_e$  then  $t$ , the clotting time will be proportional to  $C_e^{-1/2}$ .

Typical plots of  $\log(t)$  versus  $\log(C_e)$ <sup>196</sup> show that  $t$  is indeed proportional to  $C_e^\gamma$  but that the power  $\gamma$  (Fig. 14) is rarely -0.5. For human fibrinogen and milk casein  $\gamma$  is nearer to -1. Payens suggests that not all collisions will lead to particle fusion. An efficiency of  $10^{-6}$  is required in applying Smoluchowski's theory to milk coagulation, with the possible implication that only one thousandth of the area of each micelle is activated. Payens proposes that the efficiency of fusion will depend on the number of sites activated per micelle, which in turn will be proportional to  $C_e$ . If  $k_s$  and  $V$  are both proportional to  $C_e$  then  $t$  will be proportional to  $C_e^{-1}$ . A problem with Payens' theory in this form





is that it only applies for low enzyme concentrations where the enzymic reaction is zero order. Evidence suggests that a first order enzymic reaction is more commonly found<sup>198</sup>.

Substrate	Enzyme prep.	Slope	R <sup>2</sup> a)	Number of-exps.
blood plasma	lung tissue extract	0.29	0.995	18
blood plasma	chicken embryon. extract	0.41	—	—
blood plasma	chicken embryon. extract	0.50	—	—
blood plasma	chicken embryon. extract	0.68	—	—
blood plasma	thromboplastin	1.08	0.999	4
citrated horse plasma	thrombin	0.73	0.967	9
human fibrinogen	human thrombin	1.0	—	—
milk	calf rennet	0.77	0.998	18
milk	calf rennet	0.95	0.999	16
micellar casein	calf rennet	0.99	0.976	5
κ-casein	calf chymosin	0.87	0.987	4
κ-casein	M. Pusillus protease	0.83	0.992	4

Fig. 14 Double-logarithmic regression of clotting time versus enzyme dilution  
Slope =  $\gamma$

Green<sup>199,200</sup> has proposed that the theory of lyophobic colloids might apply to micelles in milk. By this theory the initial contact of micelles, leading to bonding, will depend on the balance between long range repulsive and attractive forces. These attractive forces are the London Van der Waals forces and the repulsive forces are electrostatic (being given by the  $\zeta$ -potential of the micelles). The presence of absorbed cations will reduce the nett negative surface charge on the micelles, as indeed will the removal of the anionic glycopeptide by the action of rennin, which halves the negative charge on the micelle. The reduction in electrostatic repulsive force, due to lowering the surface charge, is thought to allow the approach of micelles into close enough proximity for reaction to occur. Permanent fusion, as observed by protein bridging on electronmicrographs, is also expected to depend on the orientation and proximity of activated sites on micellar contact<sup>201</sup>. Green quotes the clotting time,  $T$ , (as measured by Koppelman<sup>202</sup> using a rotational viscometer) as being<sup>200</sup>

$$T = k/C_e + t$$

(where the first term represents the enzymic reaction time and  $t$  is the time for the secondary phase). That is, the time before initial appearance of clot is inversely proportional to  $C_e$ . In order to explain the presence of a delay time with this type of dependance on enzyme concentration Green suggests that aggregation will not occur until a minimum amount of  $\kappa$ -casein has been hydrolysed to para- $\kappa$ -casein.

Payens disputes both the relevance and the strength of these long range forces as calculated by Green. He points out that, where maximum repulsion is calculated to occur, at an interparticle distance of 0.1nm, short range forces are likely to dominate over these long range forces. Payens suggests that short range hydrophobic forces (also mentioned by Slattery<sup>203</sup>) may be more important at these distances.

Waugh<sup>204</sup> pursues the idea that multiple sites must be activated on each micelle before reaction will occur, assuming that for a gel to form there must be  $y$  areas having  $x$  neighbouring  $\kappa$ -casein units converted to para- $\kappa$ -casein and that the enzymic reaction is pseudo 1st order. Therefore according to a linear polymerisation model, as developed by Flory<sup>205</sup>,

$$y = N_0(\exp\{-k_1 C_e t\})^2 (1 - \exp\{-k_1 C_e t\})^{x-1}$$

where  $k_1 C_e$  is the rate of the enzymic reaction and  $N_0$  is the number of substrate molecules. This equation implies a rapid approach to a chosen value of  $y$  after a time delay, this time delay being inversely proportional to  $C_e$ . In this theory the diffusion time is neglected and it is assumed that there is no para- $\kappa$ -casein in the milk serum.

Flory's model has been extended by Stockmayer<sup>206</sup> and used by Peniche-Covas<sup>207</sup> to show that for multi-functional condensates in general a sudden rapid rise in the weight-average molecular weight will occur after a certain stage of reaction is reached. Since the polymer viscosity is proportional to the weight-average molecular weight this sudden increase is likely to be coincident with the onset of gelation. Parker and Dalgleish<sup>208,209</sup> have indeed used this theory to account for the lag time in heat induced coagulation of whole milk. At least three functional groups per micelle are required in this explanation.

Another interesting observation, by Cheryan<sup>210</sup>, is that milk, passed over immobilised pepsin, will clot fully regardless of whether more fresh milk is introduced that has had no exposure to the enzyme. This suggests that not all micelles need be activated by enzyme for complete coagulation to occur and that para- $\kappa$ -casein forms bonds, not with itself, but with another protein such as  $\alpha_s$ -casein. They were unable to determine the exact proportion of modified  $\kappa$ -casein that was necessary for coagulation to proceed to completion.

Comparing the observable lag phase in the milk clotting reaction with that in thrombosis, it can readily be seen that both involve the removal of acidic peptides by proteolytic enzymes<sup>81,82</sup>, their duration being inversely proportional to the enzyme concentration. Both of the delays are reduced by an increase in temperature. The secondary coagulation stage appears to be a second order reaction, dependent on pH, temperature and calcium ion concentration in both cases. Calcium is also thought to stabilise the gel structure of a fibrin clot,



and the secondary coagulation stage of milk will not occur at temperatures below 15°C<sup>211</sup>.

The reasons behind the lag phases in blood and milk coagulation may be different, although both are likely to be due in part to a difference in reaction order between the enzymic and polymeric reactions. In blood, the formation of a cross-linked mesh appears to require the removal of both FPA and FPB from the fibrinogen substrate, with the removal of FPA occurring first<sup>88,89</sup>. In experiments in which the rate of release of these fibrinopeptides is measured, a delay is apparent before any FPB is released. The delay in the coagulation of milk, on the other hand, may be due to the need to have several active sites on a micelle before collisions will lead to coagulation<sup>194,204</sup>.

Further to the observed reactions following a similar pattern, if milk is to serve as a blood analogue in clotting studies there is a need for the formed clot to adhere to surfaces and to have similar rheological properties to thrombus. Berridge<sup>212</sup> has examined the adherence of milk coagulum to various surfaces in an experiment essentially the same as that of Gott<sup>52</sup>. He demonstrated that, as with blood, the deposition of milk curd is dependent on the surface material, with Lecithin having a similar protective action to that of Heparin in the case of blood. This similarity in the material-dependent nature of both coagulation reactions is perhaps attributable to the following observation: The initial stage in the adhesion of both thrombus and milk curd appears to be the same, that of the absorption of a protein layer on the surface.

Scott Blair<sup>198,213,214</sup>, using a U-tube gelometer has shown a similarity between the rate of setting of blood, by plasma geloplastin, and milk, by rennet, and between the rheological properties of the formed clot. The rigidity modulus,  $G$ , is proportional to the logarithm of the time,  $t$ , for milk and to  $\log(t-t_0)$  for blood. The rheology of the formed clot is simpler for blood than for milk, thrombus behaving like a Maxwell body (i.e. like a single elastic spring attached in series to a single viscous dashpot) and milk curd as a Burgers body (i.e. an elastic spring and a viscous dashpot attached in parallel, connected in series with a Maxwell body). These differences in rheology may cause some variation in the behaviour of clot, especially in oscillating high shear flows. However, of possibly greater interest is the observation that the rigidity modulus of both blood and milk clots during the initial stages of clotting is similar, being in the region of 0–2000 dynes/cm<sup>2</sup>.

One advantage in the use of milk, as yet unmentioned, is that the enzyme is added in controlled amounts. This is found to remove the problem of variation in clotting power between one experiment and the next, the seasonal differences in milk composition appearing to have little effect.

#### 4.4 Previous Use of the Milk Analogue

Lewis<sup>79,80</sup> has shown that a mixture of unpasteurised milk, rennet and Calcium Chloride will behave in a similar way to blood, as far as coagulation is concerned, in a number of tests. The simplest test of blood clotting to be repeated using milk was the Lee-White test<sup>94</sup>, in which  $1\text{cm}^3$  of the mixture was made up in a 8mm i.d. test-tube. The tube was plugged and inverted every 30 seconds. The end point was taken as the time when the mixture would no longer flow. Milk behaved in a similar way to blood in this test, forming a stationary plug of clot after a finite time delay.

In a replica of Hladovec's<sup>64</sup> net experiment where  $7.5\text{cm}^3$  of the fluid, contained in a glass cuvette, is circulated by a peristaltic pump through a 50cm long, 3mm i.d. tube containing a mesh at its outlet, milk clot was observed to form downstream of the mesh (where the fluid re-entered the cuvette). This behaviour was the same as that of blood, and the corresponding pressure profile with time showed three distinct phases resembling that of blood. Lewis pursued this study to include grids of different mesh size and milk at a variety of temperatures. As in Hladovec's experiments with blood the mesh size did not appear to alter the clotting time and an increase in temperature accelerated the clotting reaction. Lewis also used a range of materials in the mesh. Clotting time did not appear to be a strong function of material type, though the quantity of adherent clot was greater in the case of materials such as bronze than for stainless steel. Furthermore, analysis of the coagulum formed revealed a similar deposition pattern to that of blood. A protein network was observed in which fat globules were trapped, the size of these fat globules increasing with distance from the mesh. The downstream clot also contained deposits of Calcium.

Lewis used this net experiment to test for the influence of Rennet concentration, Calcium Chloride concentration and temperature on the clotting time. The effect of variations in rennet and  $\text{CaCl}_2$  concentrations could be minimised by the choice of concentrations in ranges where the clotting time is almost independent of concentration. No such temperature independent region was observed, so the control of temperature was deemed to be of paramount importance.

Lewis also investigated the behaviour of milk in stagnation point flow chambers, similar to those used by Petschek<sup>66</sup>. Lewis also postulated that the first step in the process of deposition is the absorption of a protein layer on the surface with a subsequent deposition of small particles. In high shear this is sometimes followed by the deposition of large particles, in a process thought to be similar to that of the formation of white cell circle in blood. In medium and low shear it was possible to observe wedge-shaped deposits. As



with blood, wedges of milk clot appeared to be initiated at imperfections on the impingement surface. The structure of these clots revealed an increase in the size of trapped particles in the direction of flow, suggesting that the smallest particles deposit first, followed by larger particles and possibly a calcium growth if conditions are favourable. Lewis suggests that small particles, having the same adhesive force per unit area of wall contact, require a higher mainstream fluid velocity to dislodge them from the wall than do large particles, and are therefore more likely to adhere to the surface. These small particles may then modify the momentum boundary layer at the wall, allowing larger particles to deposit in the lower shear region of their downstream wake. This is in agreement with the observation that milk clots and thrombi are not observed at all in the high shear chamber. This lack of milk clot in the high shear chamber may equally be due to the fact that fluid remains for less time in the region of the wall with the possibility that the reaction will not have proceeded to the point where aggregation will occur before leaving the chamber. Lewis, in some cases, found universal clotting of milk at the rennet and  $\text{CaCl}_2$  injection point, a problem not experienced by Petschek using blood. Lewis attributed this to inefficient mixing of the reagents in this region.

Lewis then developed a single-pass milk flow system through an experimental heart chamber in order to investigate milk coagulation around prosthetic heart valves. Unpasteurised milk into which 1%vol of both Rennet and saturated aqueous  $\text{CaCl}_2$  were injected, constituted the analogue fluid. A flowrate of 30 gallons/hour and a pulsatile frequency of 70 beats/min was used for most experimental runs. The milk was heated on-line, as denaturation of proteins occurs on storing heated milk. This necessitated the use of a deaeration vessel to remove any gases evolved on heating. An artificial heart chamber, using an existing actuator<sup>215</sup>, was designed so that two valves could be tested simultaneously. Mixing of Rennet and  $\text{CaCl}_2$  was achieved by splitting the milk flow in two, injecting each reagent into half of the flow, and allowing mixing to occur in the atrial chamber of the artificial heart. Using this system, Lewis observed the formation of clot on the sewing ring and downstream of the struts with the Starr-Edwards valve, as a thin layer on both faces of the disc (though predominantly on the downstream face) and as a much larger deposit on the struts with the Bjork-Shiley valve. With the Bjork-Shiley valve coagulation in the sewing ring is not as marked.

Lewis explained the clot formed around the Bjork-Shiley valve in terms of a discrete clot around the struts and downstream of the disc mimicing the thrombus formed in blood, with a more generalised clot on the rest of the disc having no direct relevance to blood clotting. The generalised clot only appeared at high Rennet concentrations. Lewis suggested that in the case of

low rennet concentrations, milk will only clot at the most favourable sites, but that with higher concentrations of rennet a more general deposit would occur. The closest comparison between this generalised milk deposit and the behaviour of blood is that of material-related thrombus in blood. However, with milk this is unlikely to be a material-related phenomenon. It is more plausible that the shortening of the clotting time caused by the increase in Rennet concentration led to coagulating milk contacting the surface of the disc in one case and not in the other. Since the heart chamber is rigid, there is no atrial volume change during the pulsatile cycle. This leads to a larger spread of residence times in this experimental heart than might be expected in the human heart. Thus the presence of this generalised clot with milk, but not with blood, may simply be due to the degree to which the clotting reaction has proceeded before the fluid makes contact with the valve. The fact that, in general, more clot is observed on the aortic valves demonstrates that the time between initiation of coagulation and potential deposition is important. Mitral valves are more likely to remain free of clot than are their aortic counterparts. A similar effect was also produced by a reduction in the milk flowrate.

In Lewis's tests the best performance was achieved with a pivoting disc valve designed by Macleod<sup>216,217</sup>. The occluder of this valve is aerofoil-shaped in axial cross-section, enabling the valve to open fully in forward flow. A small amount of milk curd formed at the points of flow separation on both the leading and trailing edges of the disc.

The main problem that arose with Lewis's equipment was that of temperature control, due largely to the use of an overflow from his header vessel returning heated milk into the storage tank. Non-uniformity of the materials of construction of the valves also made quantitative comparisons impossible. Thus to proceed further it was necessary to redesign the equipment and to fabricate the test objects from a standard material.

In the following experiments, using a new test rig, several factors, thought to contribute to thrombus deposition, are investigated. Steady flow and pulsatile flow are also compared to ascertain whether steady flow clotting experiments, representative of conditions during systole, yield information applicable to pulsatile systems.



## Chapter 5. Apparatus

The function of the test rig is to supply either a pulsatile or steady flow of heated milk at  $37 \pm 0.2^\circ\text{C}$ , to which 1% volume of both rennet and saturated aqueous calcium chloride have been added, to the object under test. Such a milk mixture is known to coagulate fully after approximately 40 seconds, so the reagents must be added and rapidly mixed immediately prior to test section, and the residence time in the test section must be less than 40 seconds. This also naturally precludes the possibility of recycling the milk solution.

In designing the apparatus a flowrate of 5l/min was considered to be the maximum required, being the normal pumping rate of the heart (4–5l/min). This maximum flowrate was not used in the experiments for the following reason. The kinematic viscosity of milk is half that of blood. It would have been possible to increase this by addition of glycerol or high molecular weight polymer, but the action of such materials on the clotting of milk was unknown, and the expense involved seemed unreasonable. Therefore, because of this lower kinematic viscosity, the linear velocity and thus the flowrate of milk was halved, so that in experiments involving the use of standard heart valves, the Reynolds number in the region of the valve would be the same as that in the heart. The flowrate chosen for the experiments reported here was thus 2l/min, having the added advantage of halving the cost of experiments of fixed duration.

In order to keep the frequency parameter ( $\alpha = d * \sqrt{f / \nu}$ ) constant it would have been necessary to halve the frequency. However with the diaphragm pump available the maximum stroke volume obtainable was insufficient to give the required flow at this frequency, so a physiological frequency of 70 beats /min was used. This resulted in a value of  $\alpha$  40% larger than that normally found in the heart. The effect of this on flow properties is thought to be small, a variation of 20% in frequency appearing to have no influence on the overall structure of the flow in dye injection experiments.

It was thought advantageous to isolate the initial heating of the milk from the downstream test-section flow, as in Lewis's apparatus. This allows more control over the heating process, as the milk flow through the heat exchanger is steady regardless of the flow downstream, and more control over the pressure upstream of the test chamber. The latter is particularly important when using the diaphragm pump, as small changes in suction pressure result in large variations in flowrate.

The scheme is shown in Figure 15 and a photograph of the apparatus in Figure 16. In the upstream region where the flow is steady the milk is drawn

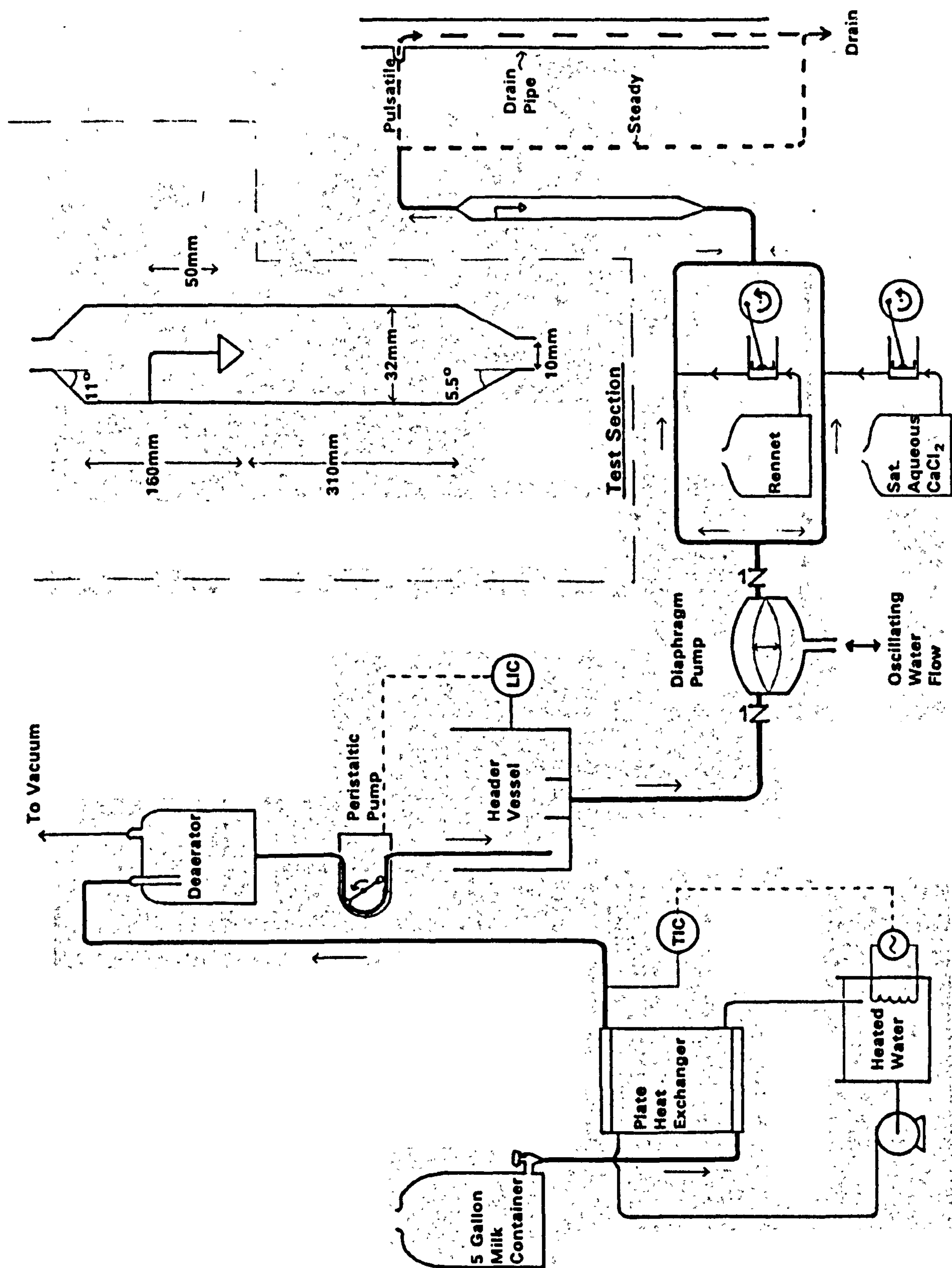


Fig. 15 Schematic diagram of apparatus



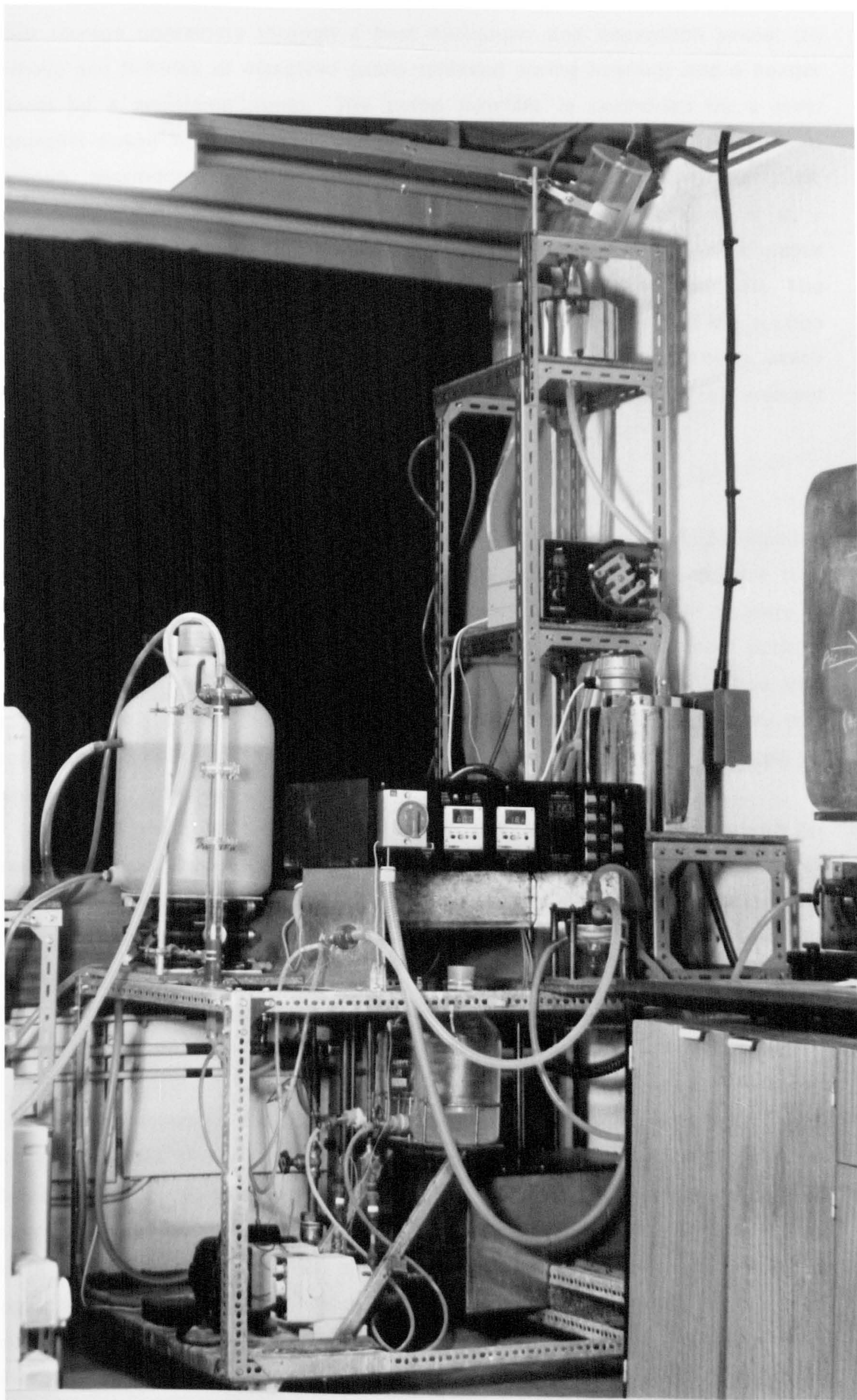


Fig. 16 Photograph of apparatus



from storage containers through a heat exchanger and deaeration vessel (to remove any bubbles of dissolved gases released during heating) into a header vessel by a peristaltic pump. The pump flowrate is controlled by a level controller linked to the header vessel, and the milk temperature is regulated through thermocouple-based control of the heater in the water tank. Downstream the heated milk, flowing either steadily under gravity or in a pulsatile manner through the diaphragm pump, has both rennet and saturated aqueous  $\text{CaCl}_2$  injected immediately prior to the experimental test-section. The downstream pressure is kept constant at a level slightly higher than the suction pressure (to prevent syphoning) by the use of a drain pipe, through which copious supplies of water flow to wash away the clotting milk. Each element of this apparatus will now be considered individually.

### 5.1 Milk Storage

Initially it was intended to use churns from which the milk would be expelled under nitrogen pressure. However, churns are no longer used and are thus difficult to obtain. The Dairy suggested that the most convenient delivery of large amounts (30 gallons) of milk would be a bag in box arrangement such as is used in hotel milk dispensers. These hold 3 or 5 Gallons each and thus appeared ideal for this duty. However, these bags in boxes are used only with homogenised milk, so, owing to the cost of this treated milk, a supply of unpasteurised milk was sought from the University farm.

Lewis had great difficulty cleaning the large tank into which he poured his milk, so it was decided at an early stage that the containers in which the milk would be collected should also serve as dispensers of milk to the apparatus. 5 gallons was considered the maximum that could be easily manoeuvred into position, so easily-cleaned 5 Gallon PVC containers were obtained, having built-in taps for dispensing the milk. Six such containers were acquired to enable storage of the 30 gallons of milk necessary for a one hour experiment.

A manifold system was designed to allow an easy change-over from one container to another. For ease of operation it was thought best that at least three containers should be capable of being linked to the system at one time. Since laboratory space was limited, a manifold system for a maximum of three containers was thus chosen. This is shown schematically in Figure 17. A water tank was also included, since the apparatus has to be heated up to operating conditions using water before exposure to milk. A double valve system for each milk container was necessary to allow for disconnection of the milk containers. At one stage a spring-loaded container support system was considered so that the head of milk would remain constant as the milk was used. However, using the peristaltic pump this was deemed unnecessary.



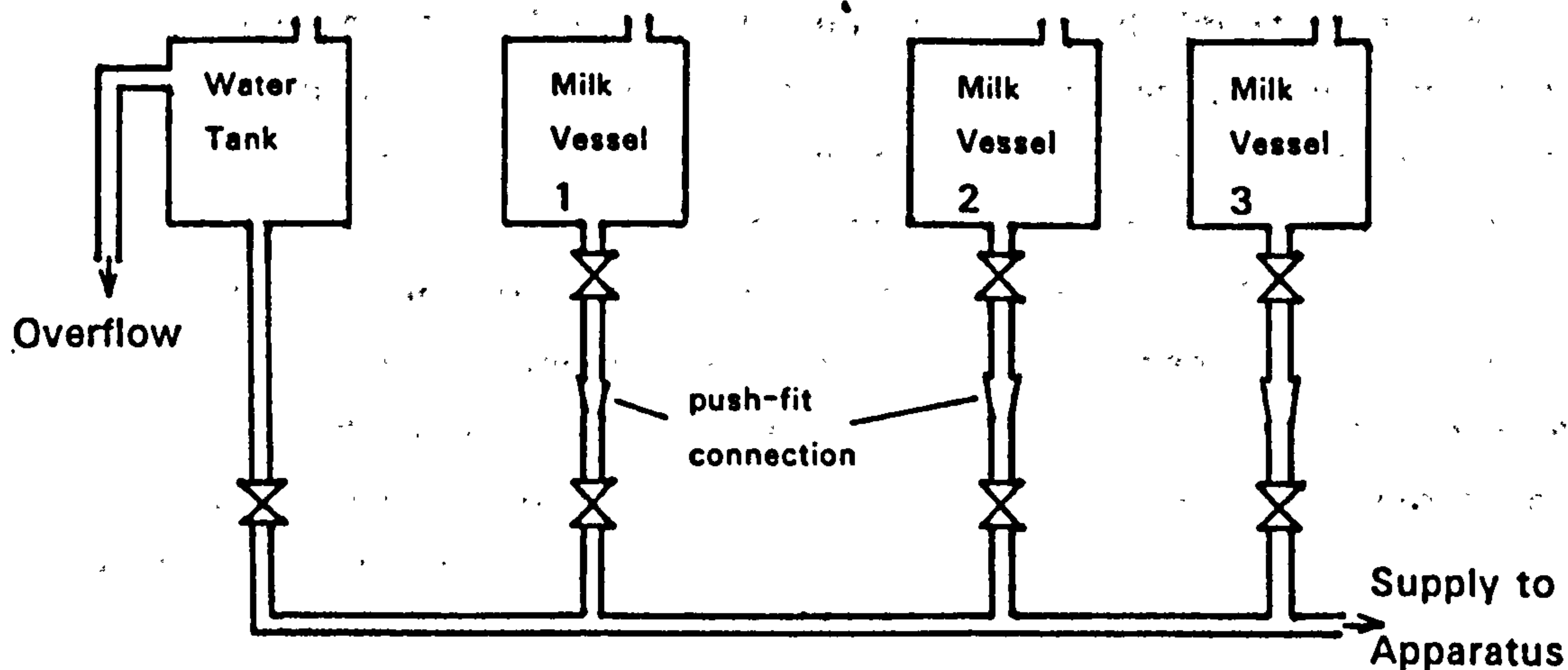


Fig. 17 Schematic diagram of manifold arrangement

## 5.2 Pump on upstream side

As mentioned previously, at a fairly early stage a decision was taken to separate the preparation of heated milk from the downstream flow since this allows steady flow through the heat exchanger and maintenance of a steady pressure in the deaeration vessel.

Lewis had problems with a skin forming on the surface of the milk in the storage tank. It was thought that this could be eliminated by having the milk in contact with  $N_2$  rather than air. Blowing the milk through the system under nitrogen pressure was thus considered. This would have the second benefit of removing the need to expose the milk to high levels of shear in a pump. However, since milk churns were not available as suitable pressure vessels, this idea was discarded. It transpired, in practice, that the problem of skin formation was not observed.

The simplest alternative means of milk delivery was to use a peristaltic pump with feedback from a level controller in the header vessel. A Watson Marlow 603U/R peristaltic pump with 12.7mm i.d. tubing, having a maximum flow of 8l/min at low suction pressure, was chosen for the purpose.

This was originally positioned upstream of the heat exchanger and deaerator but due to the compliance in the deaerator (caused by the use of a water ejector to draw provide a vacuum) the response to the level controller was poor and severe problems were presented in maintaining a constant pressure in the deaerator. A sudden change in milk delivery would either draw water into the deaerator or allow milk to be ejected into the water flow, thus preventing the change in pump speed from having an immediate effect on the level in the header vessel. The pump was therefore moved downstream of the deaerator. This provides its own design problems in that a large suction

pressure is now required. The peristaltic pump is situated almost 6ft above the milk storage containers, giving a static pressure approximately 150mmHg below atmospheric pressure. Taking into account the losses in the rubber tubes and the heat exchanger, and using the manufacturers' data for the variation of flowrate with suction pressure, a maximum flowrate of 7.0l/min was still expected. However, in practice, no flowrate greater than 4.0l/min is then attainable. This did not provide problems in the current study as no flowrate greater than 3l/min was required, but if *in vivo* blood flowrates were to be obtained a different pump would be necessary.

### 5.3 Heat Exchanger

A plate heat exchanger<sup>218,219</sup>, such as is used in the dairy industry, was chosen for ease of cleaning and because of the high heat transfer co-efficients attainable. The heat duty required to raise the temperature of 5l/min of milk from about 5°C to 40°C is 11.5kW. This was to be achieved by way of a secondary hot water circuit, the temperature of which would need to be kept to less than 15°C above that of the heated milk to prevent fouling. A value of 45°C at the inlet was therefore chosen to prevent protein denaturation at the plate surface. Also, to facilitate temperature control, a high water flowrate relative to the milk was chosen (25l/min) giving a water outlet temperature of 35.2°C. Using typical heat transfer data from Flack<sup>218</sup>, for overall heat transfer co-efficients in plate heat exchangers with 2 water flows (i.e. 4000W/m<sup>2</sup>K - 5700W/m<sup>2</sup>K), it was decided that a value of about 3-4000W/m<sup>2</sup>K might be reasonable for a water/milk system. Using this data a heat transfer area of about 0.2m<sup>2</sup> appeared reasonable. This indicated the use of an APV Junior Paraflow heat exchanger, where the individual plate area is 0.025m<sup>2</sup>. For ease of assembly a parallel flow single pass system was chosen. The final design supplied by APV is a single pass system with 6 parallel water channels alternating with 5 parallel milk channels. (The plate dimensions, etc., are given in Appendix A). The exchanger is fabricated from 316 stainless steel with Paracril gaskets to minimise fouling by milk proteins.

As mentioned briefly before, the flowrate on the water side is maintained at a high level to minimise the delay between altering the power supplied to the water heaters and the temperature change in the exchanger. A single-phase 2kW and 3-phase 4.5kW and 6kW heaters are used to heat the water, with the temperature control being effected by an Anglicon CGPIDT temperature controller acting on one phase of the 6kW heater. A switch on the 4.5kW heater supply and a switch on another phase of the 6kW heater served to reduce the heating power in the event of reduced flowrates.



#### 5.4 Deaerator

In the process of heating milk from 5 to 40°C dissolved gases are released from the milk. If these small bubbles of gas are not removed they may provide nucleation sites for clot formation. A 0.5 to 1 minute hold-up in the deaeration vessel was considered sufficient for bubble disengagement, using ceramic balls to aid this process. A 5 litre insulated stainless steel container 3/4 full of ceramic balls was therefore used for deaeration with approximately 6ft of water vacuum being applied via a water ejection nozzle. Two 7 litre perspex containers were connected into the vacuum line, as shown in Figure 18, to provide capacitance on both the milk and water side. This prevents mixing of the milk and water in the event of either a sudden rise or fall in pressure from either a change in pumping action or the change-over of milk containers.

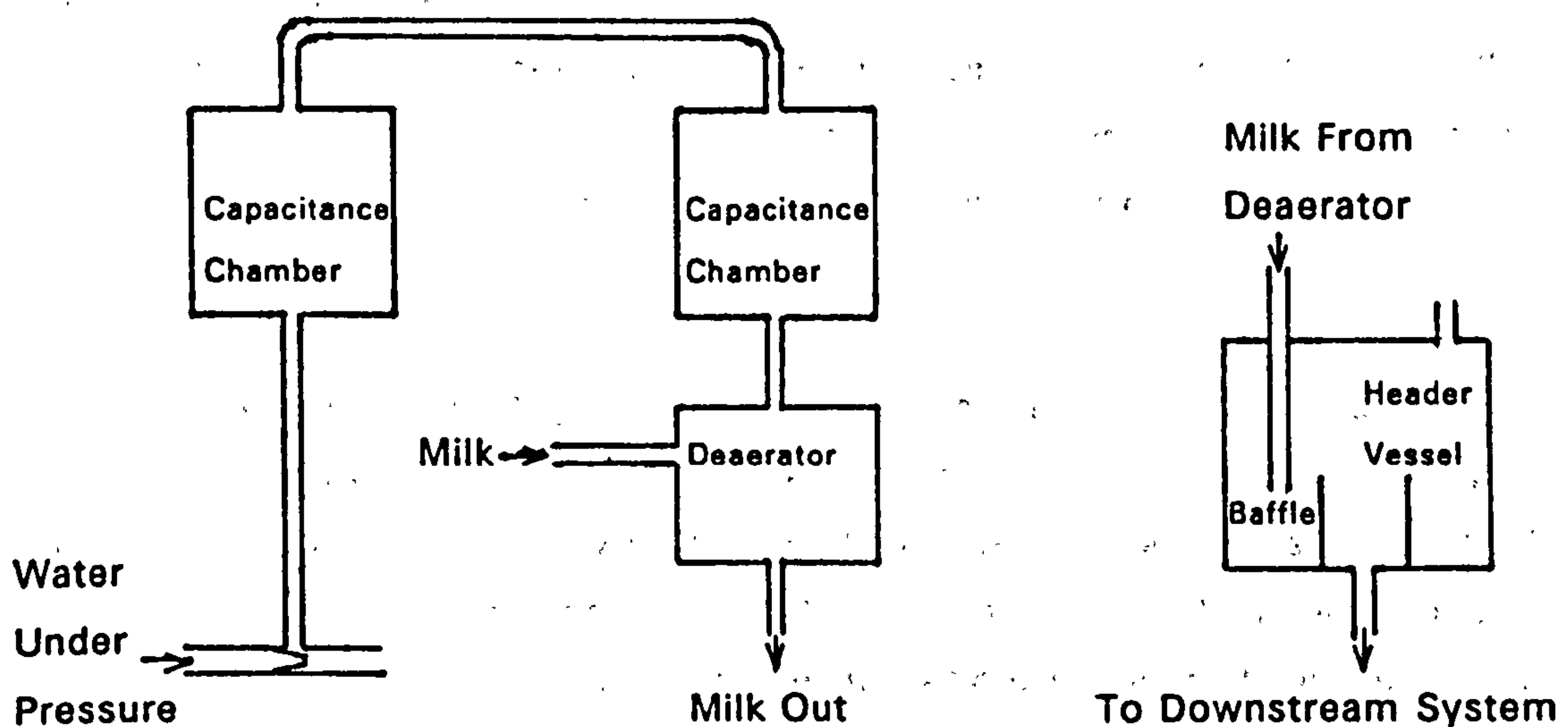


Fig. 18 Schematic diagram of deaerator system and header vessel

#### 5.5 Header Vessel and Level Control

A 5 litre insulated stainless steel container with a hold-up time of up to 2 minutes was chosen to provide a supply of heated milk at constant head to the downstream test section. A diameter of 20cm was chosen so that fluctuations in level due to the pulsatile nature of the flow should be no more than a few millimeters.

Level control is effected using a Telstor Series 80 level sensor and controller, the signal from the controller being used to activate the Watson Marlow peristaltic pump. A baffle was placed in this vessel (Fig. 18) to encourage any large bubbles of gas, carried through from the deaerator, to disengage before the milk is drawn into the test section.

## 5.6 Downstream Pumping System

The diaphragm pump, first used by Knight<sup>215</sup>, provided a pulsatile flow whose frequency ( $20\text{--}150\text{min}^{-1}$ ), stroke volume ( $0\text{--}100\text{ml}$ ), and systolic/diastolic duration ratio ( $0.4\text{--}2.0$ ) could be independently varied between the limits indicated in brackets. In all the pulsatile flow milk experiments reported here the frequency was set at  $70\text{ beats/min}$ , the stroke volume at about  $30\text{cm}^3$  and the systolic/diastolic duration ratio at 1.

Two Liquid Metronics Series A1 metering pumps, with frequency range  $15\text{--}100\text{min}^{-1}$  and whose stroke volumes could be varied between  $0\text{ml}$  and  $0.63\text{ml}$ , giving a maximum flowrate of  $0.063\text{l/min}$ , were used to provide  $1\%\text{vol}$  injections of both rennet and saturated  $\text{CaCl}_2$  into the main milk flow. These were set at a frequency of  $70\text{ beats/min}$  and a stroke volume of  $0.3\text{cm}^3$  for the pulsatile flow experiments.

The same injection pumps were used in steady flow, with the milk flow being allowed to drain through the system under the influence of gravity. Flowrates of both  $2$  and  $3\text{l/min}$  were used in steady flow experiments.

## 5.7 Mixing System

Due to the fairly short lag time in the milk clotting process it was essential that uniform mixing of the rennet and  $\text{CaCl}_2$  with milk was achieved rapidly. In Lewis's apparatus the milk flow was divided, with rennet being injected into half of the milk and  $\text{CaCl}_2$  into the other half. The flows were then recombined in the atrial chambers of the experimental heart, where swirling of the fluid would ensure rapid mixing.

In this apparatus, back-mixing is to be minimised so that the history of the fluid in any part of the flow may be determined. Therefore a means of providing uniform mixing of pulsatile injections in a pulsatile plug flow regime was required. Two systems were developed for this purpose:-

(i) A Chemineer Kenics KMS static mixer was initially proposed for this mixing duty.  $8\text{mm}$  i.d. tee-pieces were provided by the manufacturer of the apparatus to link the injected flow with the main milk flow. These were replaced with axial injection nozzles of  $1.5\text{mm}$  bore with  $\text{CaCl}_2$  being injected at a point  $6''$  upstream of the rennet, since tee-pieces would allow milk to enter the injection system leading to coagulation in this region.

It was not possible to link the phases of the injection pumps with that of the diaphragm pump for the milk. Dye injection experiments were therefore conducted to assess the uniformity of mixing of the injected reagents with the main flow. These indicated that radial mixing was excellent with this system, as expected with a static mixer, but that axial concentration variations were present.



This injection system was further tested to ascertain whether axial mixing in the static mixer would be sufficient to disperse the components evenly throughout the milk flow. A conductivity probe was used to determine the uniformity of mixing of an electrolyte with a water flow for a range of injection and mainstream pulsatile flows. The results of this study were presented at the 5th European Conference on Mixing<sup>220</sup> (a copy of this paper is included as Appendix B). Two aspects of the work contained therein are in hindsight unsatisfactory: no control experiment, without the use of a static mixer, was provided, and the dimensionless groups chosen should have included the standard frequency parameter  $\alpha$  ( $=d\sqrt{f/\nu}$ ). It can easily be seen, however, that  $\alpha$  is included in the pulse number  $N_p$  (better expressed as the ratio between the residence time in the static mixer and the period of oscillation of the injection pulse) since  $N_p = \alpha N_{LD}/Re$ . Regarding the former criticism, at the time of the experiments the static mixer was cemented into the flow line. Thus it could not easily be replaced by an empty flow section. Further investigation, with the assistance of two students, Robert Leask and David Lumsden, recording temporal variations in conductivity, has revealed that axial dispersion, caused by the pulsatile nature of the main fluid flow, will provide the same measure of longitudinal mixing in the absence of the static mixer as when the static mixer is present. Thus the relationships indicated in the paper are a property of the pulsatile flow and the gross dimensions of the pipework between the injection point and the conductivity cell, not of the nature of static mixer. The conclusions of the paper hold, however: The best performance will be achieved when the frequencies of the injection pumps and the main flow peristaltic pump are the same, regardless of phase differences between them, so long as the fluid has travelled for a time downstream equivalent to twice the period of oscillation of the injection pulse. Thus, for physiological pulsatile frequencies, adequate mixing will occur in a straight pipe system within 2 seconds, even without a static mixer.

(ii) Since the inclusion of a static mixer offered no significant advantage, it was removed from the flow system, thus lowering the overall pressure drop, leading to an improved performance from the diaphragm pump. In order to improve radial mixing and to prevent high concentrations of activated rennet locally in the flow, a system similar to Lewis's is employed. The milk flow is divided equally in two by a "Y" junction with rennet being injected into one limb, saturated aqueous  $\text{CaCl}_2$  into the other. The flows are then recombined in another "Y" piece immediately prior to entering the test section. This mixing system is used in all the milk experiments reported here.

## 5.8 Test section

Since many researchers had commented on the relationship between stasis and thrombosis, and since it is known that the milk will clot *en masse* after a known time delay, it was considered essential to provide a system with plug flow behaviour upstream of the test object. A cylindrical test section was therefore chosen as being the most appropriate for the purpose, pulsatile flow giving the added advantage that, even at Reynolds numbers indicative of laminar flow in a steady regime, a plug-type flow is often achieved. This was found to be the case in dye injection experiments.

It was intended to use a Bjork-Shiley valve in two of the experiments, so a pipe diameter of 30mm was chosen, the pipe being made from perspex to facilitate residence time and laser doppler anemometry experiments. The test chamber was fabricated in flanged sections so as to allow variation of experimental arrangements and to allow easy access to test objects following an experimental run (See Fig. 19 for the dimensions of the flanged sections). The flexible silicone rubber tubing used to connect the apparatus together was 12.7mm i.d. so that the flange at the inlet of the test chamber had to incorporate a diffuser, to avoid stasis downstream of the expansion. The diffuser was also important in allowing the flow to become stable at distances of only a few pipe-diameters downstream of the entrance. This, as will be seen in the next chapter, was important in order to keep the bulk residence time of the activated milk upstream of the test-body less than the Lee-White clotting time. A cone half-angle of  $5.5^\circ$  was used, this being the smallest angle able to be machined with conventional tools in a 5" long internal cone.

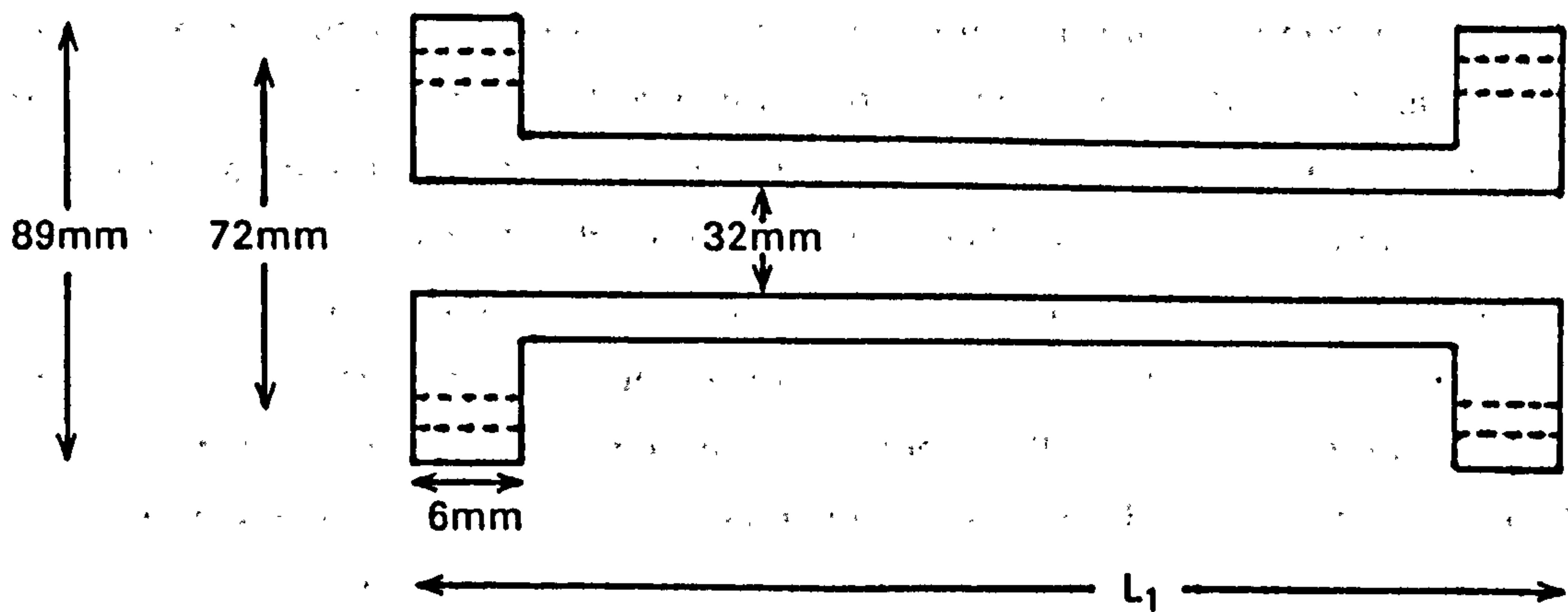
At the exit of the chamber a converger was used, an angle of  $11^\circ$  being considered satisfactory, as little backflow was anticipated. The sole purpose of this converger was to prevent pockets of fluid remaining in the exit region.

The test objects will be discussed in the next chapter.

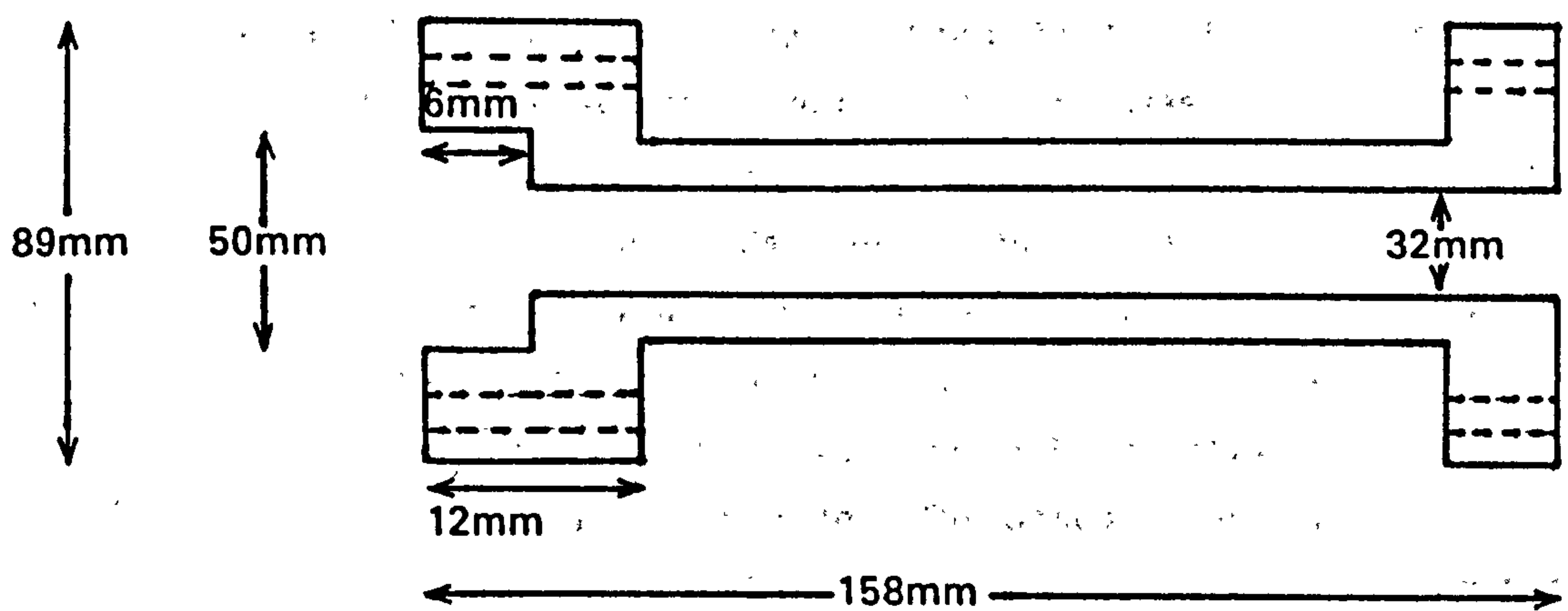
## 5.9 Waste Disposal

For pulsatile flows the outflow from the test chamber was discharged into a 2.5" i.d. drainpipe at an elevation of 1ft above the level of the header tank. Water was forced to flow down the inside walls of this pipe by a conical flow distributor, a hole being left in the centre of the cone to enable air to enter the system through the top of the pipe. Copious supplies of water were used to flush the milk coagulum down the sink. In the case of steady flow experiments the tube from the test chamber discharged directly into the sink, the elevation of the discharge point being used to control the flowrate. Again the coagulum was flushed away with copious amounts of water.

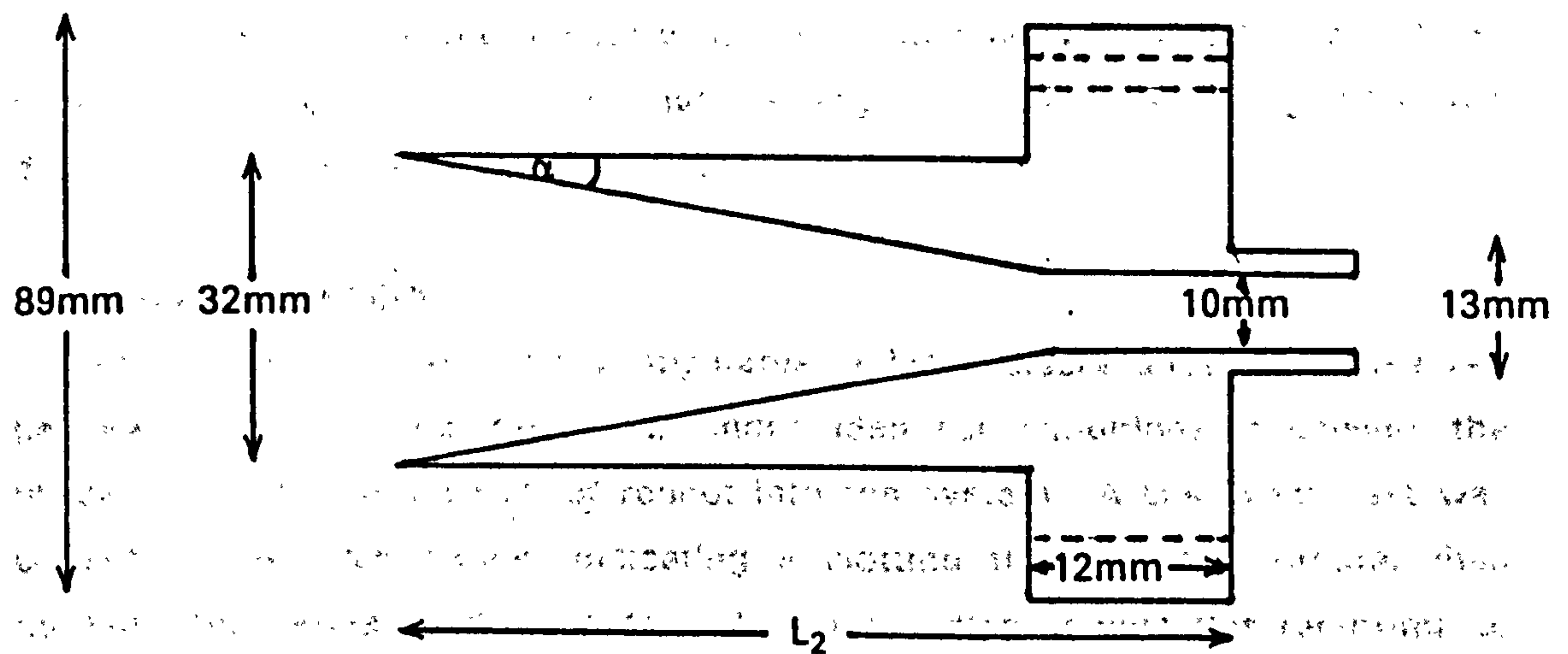




a) Three plain perspex pipes with  $L_1 = 155, 306$  and  $776\text{mm}$ .



b) Perspex pipe with recessed flange.



c) Diverger ( $L_2 = 111\text{mm}$ ,  $\alpha = 5.5^\circ$ ) and Converger ( $L_2 = 56\text{mm}$ ,  $\alpha = 11^\circ$ ).

Fig. 19 Diagram of flanged sections

## 5.10 Operation

The test section is assembled with the appropriate test object in place, and air is flushed out using water. Three of the milk storage containers are then connected into the manifold system. The apparatus is brought up to the operating temperature using water, supplied from a constant head tank. Any air bubbles that adhere to the test chamber walls during the heating process are dislodged percussively and the water flow is then replaced by a milk flow. Details of the operating technique are reproduced in Appendix C.

During the course of the experiment, as each milk container empties another is brought on-line, the empty container being replaced by a full one. Once the last milk container is empty, water is reintroduced into the system. When the water, or diluted milk, starts to enter the header vessel, the peristaltic pump and both injection pumps are stopped. The remaining milk in the header vessel is used to flush out the enzyme-activated milk still in the test chamber.

The test chamber is then slowly drained from the top down, by loosening each flange in turn. This prevents adhering clot being washed away, either by bubbles rising through the test chamber or by a rapid efflux of milk. The latter problem has to be avoided in particular, due to the formation of a thin layer of clot on the walls of the chamber in all experiments. If this clot became dislodged, it could brush off all the clot adhering to the object itself.

Once the object is removed it is weighed and photographed. The test chamber is initially cleaned using hot water. The upstream flow section is then connected as a continuous recycle by inserting the outflow of the peristaltic pump into the water storage tank (drained to 1/5 capacity) and likewise the downstream flow section by reconnecting the tube formerly leading to the drain into the entry to the header vessel. 0.1M Caustic Soda (ODC) is circulated in the upstream loop and 1%wt aqueous tergazyme (enzymic detergent) in the downstream loop where clotting occurs.

## 5.11 Enzyme Strength

During commissioning of the apparatus, a large discrepancy was observed between one run and the next, under identical conditions, following the introduction of a new batch of rennet into the system. A Lee White test was carried out on this rennet, indicating a clotting time 3 times greater than normal. This rennet was discarded. As a precaution against this recurring, or indeed against the quality of milk varying, a Lee White test at a set temperature of 33.5°C was carried out prior to each experimental run. This lower temperature for the Lee-White test, compared with that of the milk run, was used to prolong the clotting time, so that any variations in this time would be more noticeable.



## **Chapter 6. Testing of Axisymmetric Bodies and Valves**

The aim of this experimental work was to provide a standard reproducible test for coagulation and to attempt to discriminate between several hydrodynamic factors thought to be responsible for thrombosis. The factors chosen for investigation, based on an analysis of Hladovec's net experiment<sup>64</sup>, were stasis and turbulence. In order to be able to isolate individual or simple combinations of flow factors, axisymmetric test pieces were employed throughout (except in the testing of the Bjork-Shiley valve) and the test section was designed to promote plug flow behavior. The intention was to duplicate Vorhauer's experiments using various shaped bodies of revolution and Gott's experiments with a vena caval orifice-containing ring<sup>62,63</sup>. Several of these experiments proved infeasible with this apparatus for reasons which will become clear later.

A further set of experiments, using the Bjork-Shiley valve, was also conducted to attempt to determine whether deposition in the region of this valve occurs during systolic flow or whether the deposition pattern is indicative of conditions of diastole.

The use of Vorhauer's test bodies will be discussed first, followed by experiments with the Bjork-Shiley valve and finally a discussion of the limitations of the apparatus and consequent need to modify the test programme revealed by these experiments.

### **6.1 Vorhauer's Test Bodies**

Vorhauer<sup>68,120,121</sup> carried out an elaborate set of experiments in which a number of bodies of revolution (7.6mm diameter), supported individually on metal stings, were implanted in the canine descending thoracic aorta (10mm diameter). In his first set of experiments, each object was mounted by a metal sting screwed into its downstream end (Fig. 4). The thrombus adherent to the body itself was weighed after these 45 minute tests. Vorhauer found that the larger the wake region and the more intense the wake turbulence (as measured in a model aortic test rig using birefringent fluid) the greater was the quantity of thrombus deposited. Thus the objects in order of increasing thrombogenicity were the tear-drop (cusp downstream), sphere, disc, downstream-apex cone, and upstream-apex cone.

In a second set of experiments, aimed at deducing the thrombogenic effects of free-stream turbulence caused by the presence of the test bodies, these were mounted in turn on a metal sting at the forward stagnation point (Fig. 5). The weight of thrombus on the mounting sleeve (downstream of the test

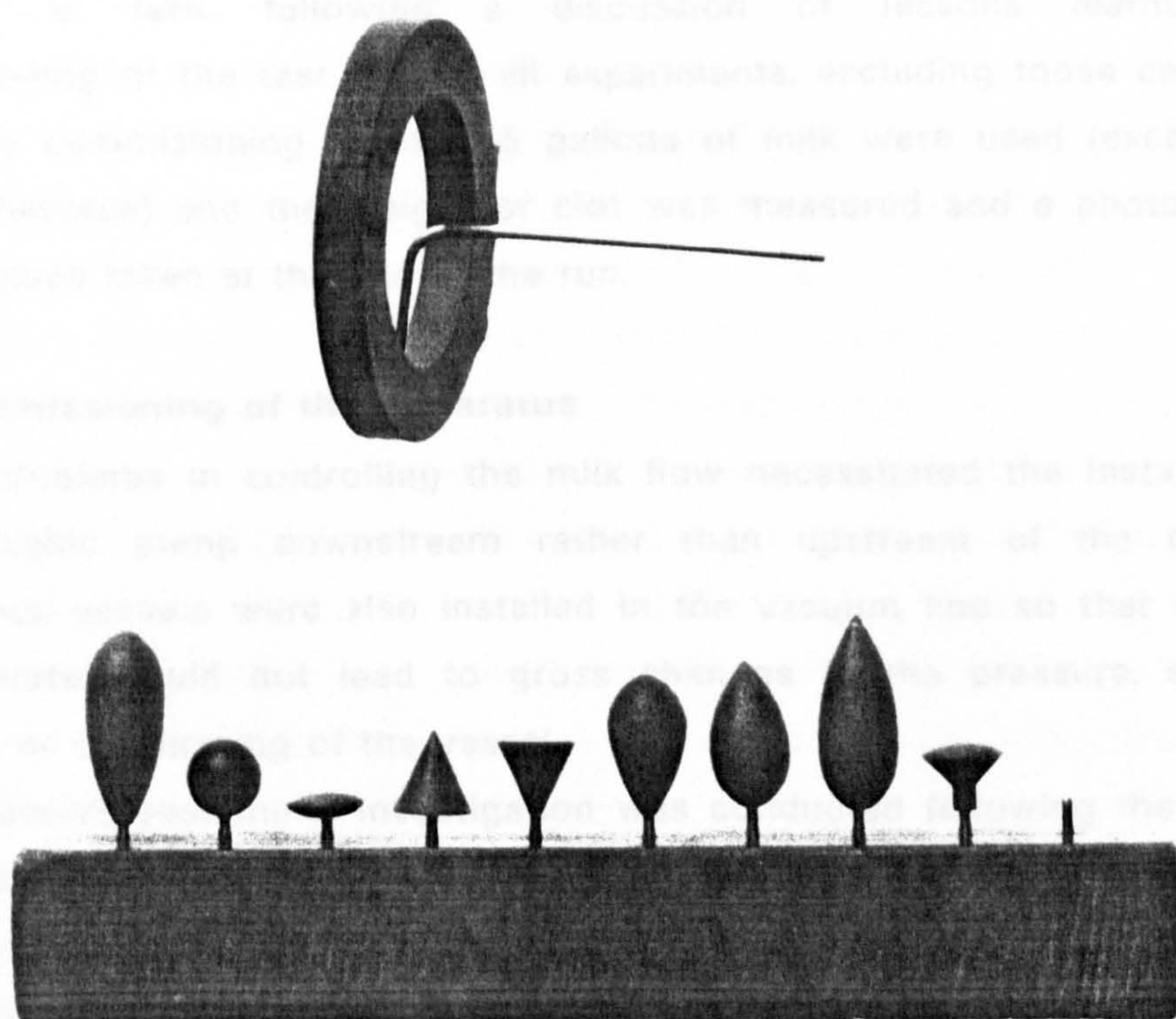
bodies) was measured for each after 25 minutes. The shorter time was chosen to prevent the possibility of clots forming on the bodies becoming dislodged and collecting on the mounting sleeve. A surprising observation was made: With all but the 'lunar module, re-entry capsule'-shaped test-piece the weight of thrombus deposited in the sleeve was less than with no test body present. Vorhauer attempted to measure the turbulence power spectra in the region of the mounting sleeve by means of dynamic wall pressure measurement in his mock aortic flow apparatus. By calculating the area under the spectrum curve and using this as a measure of turbulence intensity, he found that in order of increasing free turbulence the bodies were tear-drop, sphere, and disc, with the cone varying in relation to the others depending on the flowrate. The cone lies between the tear-drop and the sphere at low stroke volumes and after the disc at high stroke volumes. The ordering of these objects with regard to increasing thrombus in the mounting sleeve was tear-drop, cone, sphere and disc, coinciding with the ordering of free turbulence at low stroke volumes. However, only the tear-drop produced less turbulence than did the empty test section, and even then only at the lowest stroke volume. The reduction of clot in the sleeve when the test bodies are present is not, therefore, simply a consequence of some reduction of turbulence.

Vorhauer concludes that the size of thrombi produced downstream of the objects varies both with the size of the region of stasis and with the intensity of the wake turbulence (The proportion of red blood cells in the thrombus also decreases as the shear stress increases.). He proceeds to recommend that in the design of prosthetic heart valves, sharp corners, abrupt changes in cross-sectional area, blunt bodies and surfaces exposed to slow-moving blood should all be avoided. In fact, Vorhauer's experiments do not fully differentiate between thrombus deposition induced by various sizes of regions of shear and that induced by intensity of turbulence. In the first set of experiments, both stasis and turbulence are present in the wake of the objects. In the second set, whilst only turbulence is present in the region of the mounting sleeve, effects on fluid in this region due to stasis in the wake of the objects cannot be discounted. His second set of experiments show, to a certain extent (though the uncertainty in the ordering of the objects precludes a definitive assertion) that turbulence may have an influence on the degree of thrombosis, but in the first set stasis is not necessarily directly implicated since either turbulence or stasis could be responsible for determining the quantity of thrombus deposited in the wake region. It is also possible that stasis alone is responsible for deposition in both experiments, with either carry-over of clotting material from the wake region, or stasis in the boundary layer at the vessel wall, being responsible for clotting in the mounting sleeve. Thus whilst



both stasis and turbulence may be contributing to thrombus formation in these experiments, it is possible that stasis may be solely responsible for the weight of thrombus deposited. However, in the light of the discussion above, it is evident that turbulence alone cannot account for the size of deposit.

For the milk experiments, bodies of revolution of the same shape as those of Vorhauer were machined from PVC. A hole was drilled in the downstream end of each object so that a push-fit mounting on a stainless-steel sting could be achieved. Figure 20 shows a photograph of the test bodies and mounting sting (Only the five shapes to the left of the photograph were used in these experiments.). Difficulty was experienced in obtaining Vorhauer's thesis in which the linear dimensions of his test bodies are recorded. Therefore, the objects for use in these milk experiments were fabricated to be 10mm in diameter so as to occlude only 10% of the flow channel. From Vorhauer's thesis it later became apparent that his implanted objects occluded more than 50% of the canine aorta, the flow around them thus being strongly influenced by the presence of the constraining walls.



**Fig. 20** Photograph of Test Bodies and Sting

In all of the experiments reported here, the test piece was mounted on a sting, the sting being attached at its downstream end to a PVC ring. This ring was inserted into a recessed flange at the downstream end of a 158mm long perspex pipe, this flange being bolted to that of another pipe section of equal length containing an  $11^\circ$  convergence nozzle at its downstream end



(Figs. 19 & 20 ). Three lengths of test-section were utilised; long, medium and short, depending on the attachment at the upstream, or inflow, end of this test-piece mounting double-pipe section.

Early experiments employed the long, 776mm, pipe section linking the 5° diffuser to this double-pipe section. This long test section was originally deemed necessary to allow stabilisation of the flow upstream of the test-piece. Dye injection experiments later revealed that this is not required. In this long test chamber the milk encounters the test object 21 seconds after the addition of rennet and  $\text{CaCl}_2$ . Dye injection experiments in the empty test chamber show that the spread of residence times is very small over all but a thin layer next to the pipe wall.

Later experiments involved a medium length chamber and a short chamber. The medium-length chamber, with a 306mm pipe replacing the 776mm one, gives a 10 second mean residence time of the reaction mixture upstream of the test object, and the short chamber, with the diffuser connected directly into the test-body mounting section, gives a 2.5 second residence time.

The tests carried out with each of these test section lengths will be discussed in turn, following a discussion of lessons learnt during commissioning of the test rig. In all experiments, excluding those carried out during the commissioning period, 15 gallons of milk were used (except when stated otherwise) and the weight of clot was measured and a photograph of the test-piece taken at the end of the run.

### **6.1.1 Commissioning of the Apparatus**

Initial problems in controlling the milk flow necessitated the installation of the peristaltic pump downstream rather than upstream of the deaerator. Capacitance vessels were also installed in the vacuum line so that surges in milk flowrate would not lead to gross changes in the pressure, or indeed emptying or overflowing of the vessel.

An extensive systematic investigation was conducted following the first use of the apparatus with milk, when the bulk milk flow was found to coagulate throughout the whole test chamber.

The ingress of vapour bubbles into the system was one problem thought to be contributing to this bulk coagulation. Several modifications were incorporated to minimise this problem:-

(i) As mentioned above, capacitance chambers were installed in the vacuum line to prevent the deaerator from emptying or overflowing due to surges in the milk flowrate. This overcame much of the problem of air being entrained with the flow into the header vessel.



(ii) A baffle was installed in the header vessel to prevent passage of bubbles of air entrained in the flow from the deaerator, from entering the line to the test chamber.

(iii) The test section was installed vertically rather than horizontally, as at first, with the exit uppermost to assist the flow of any entrained air through the system and out to the drain.

Stasis, upstream of the test object, was also considered to be a problem. The installation of a diffuser at the entrance, and a converger at the exit, of the test chamber, and the removal of the static mixer, were found to remove much of the problem of gross clotting of the flow.

The temperature of the milk was also reduced from 40°C to 37°C when using all but the shortest length of test chamber.

One interesting phenomenon, discovered early in the experimental programme, was that the clot adhering to the wall of the test chamber appeared to form first at the downstream end of the test chamber and then to propagate upstream. In one instance, in an experiment using only 5 gallons of milk with no test object in place, a fairly thick 'V'-shaped clot was found on the wall of the chamber. A uniform layer of deposit, less than 0.5mm thick, was observed elsewhere in the test section. This V-shaped clot was similar to the wedge clot, reported by Lewis in his replica of Petschek's stagnation point flow chamber. However, this V-shaped clot pointed downstream rather than upstream, as was the case with the wedge clot. It was thus considered possible that coagulation at the walls might be occurring preferentially during diastole. Regurgitation on valve closure was found to be only 1.5cm<sup>3</sup>, or 5% of the forward flow. It seemed doubtful that such a low backflow might be responsible for such pronounced propagation upstream. It is possible though, that if prolonged contact time of activated fluid with a surface is essential for deposition, then diastole will indeed provide more favourable conditions for deposition. Similar V-shaped clots were observed in some other 5 gallon milk experiments.

Testing of a Tear-drop shaped object in the flow using 5 gallons of milk resulted in clot forming preferentially in the wake of the object. Clot forming on the upstream face of the object appeared only to form around bubbles of air adherent to this surface.

Once testing was complete, the experimental duration was increased to about 35 minutes by the use of 15 gallons of milk. This was deemed the shortest experimental duration that would give reproducible run times. Start-up and shut-down lasted between 1 and 3 minutes. Thus a run time of approximately 12 minutes with 5 gallons of milk could vary by 17%, whereas

only a 6% variation was expected using 15 gallons. Lewis used 30 gallons per run for reproducibility but, owing to time constraints and cost, this was considered excessive.

Initial tests, passing 15 gallons of milk over the disc in the long test chamber, indicated that an adequate level of reproducibility in the quantity of coagulum deposited was being achieved, except for one instance in which the rennet was found to be less potent than usual (see section 5.11 for details). The results from 5 consecutive runs, excluding those with impotent rennet, are presented in Table 1. The variation in the weight of clot downstream of the object, for instance, is less than 20% of the mean. Thus it was decided, for reasons of time and cost, that each object, at least initially, need only be tested once.

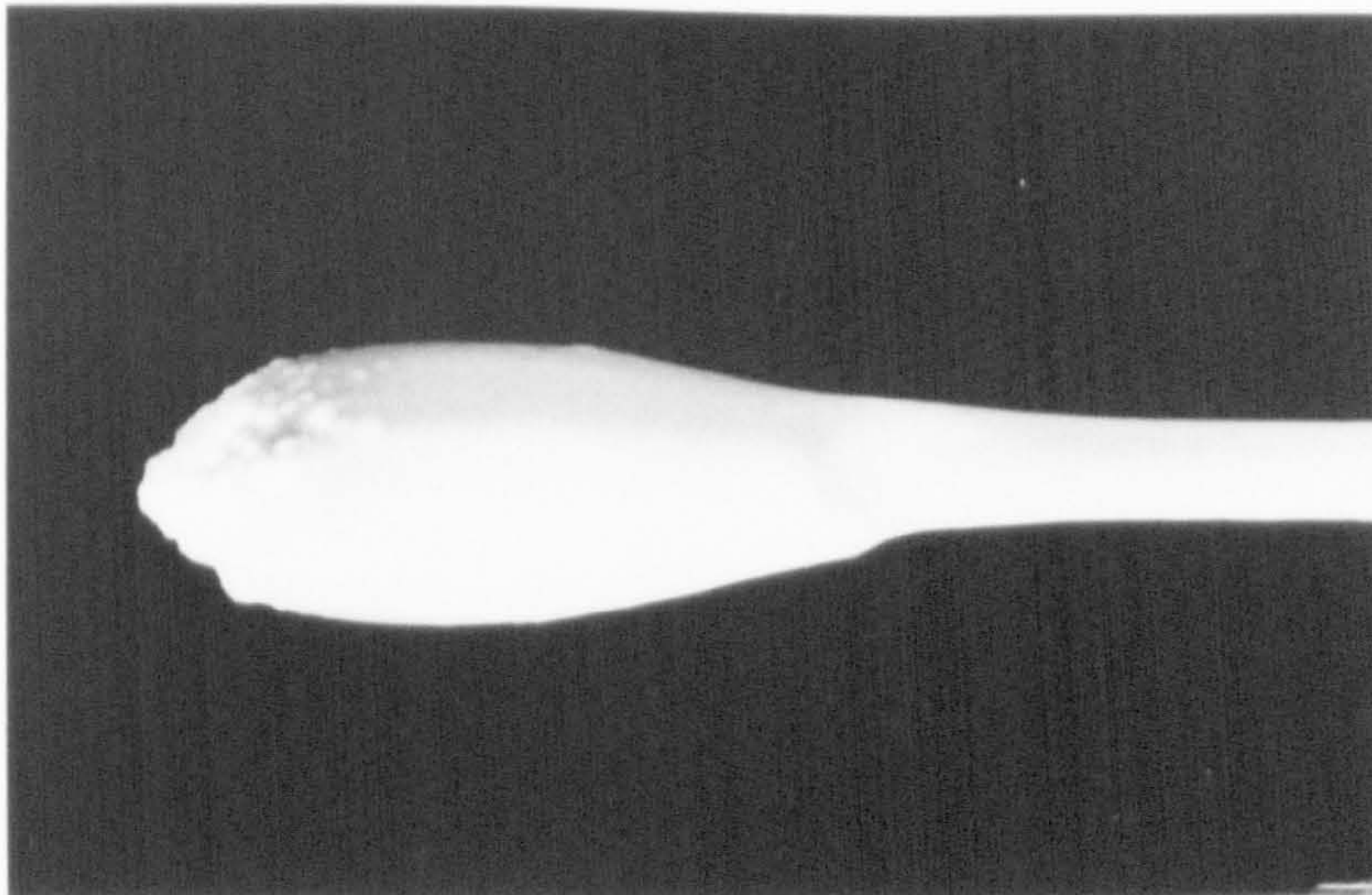
**Table 1**  
**Reproducibility of Weights of Clot Deposited Around the Disc**

Run Number	Wt. Upstream Clot / g	Wt. Downstream Clot / g	Wt. Total Clot/g
1	0.70	0.70	1.40
2	0.54	0.98	1.52
3	0.59	0.88	1.47
4	0.70	0.72	1.42
5	0.51	0.73	1.24
average	0.61 (SD=13% )	0.80 (SD=14% )	1.41 (SD=7% )

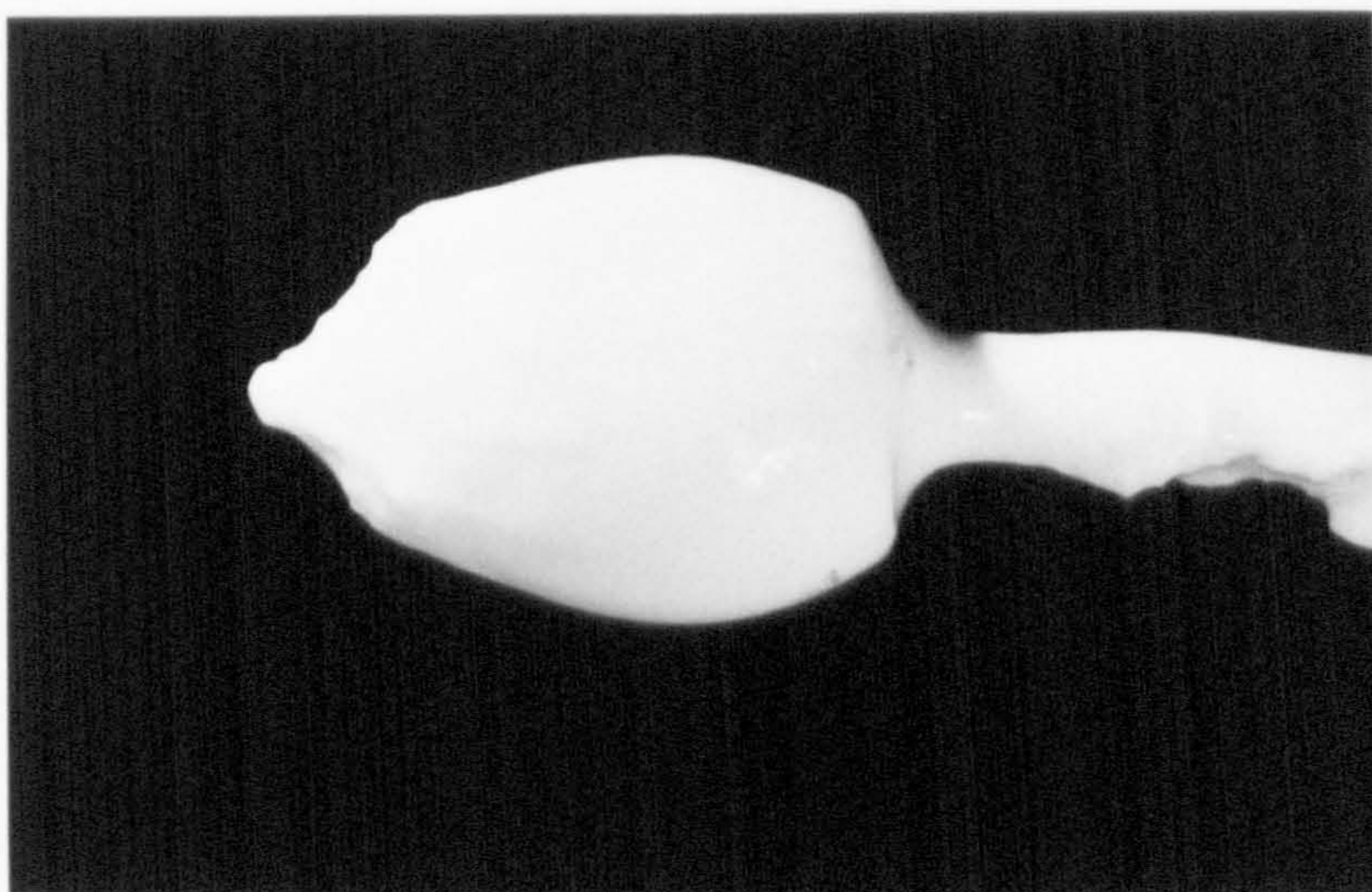
### 6.1.2 Long Test Section

Three different tests were performed in this chamber. In the first, the deposition of milk curd was measured around four of the test pieces (tear-drop, disc, sphere and upstream-apex cone) for a pulsatile milk flow. The flowrate in all cases was 2l/min and the pulsatile frequency  $70 \text{ min}^{-1}$ . Photographs of the objects and adherent clot are shown in Figure 21 and the weights of clot are recorded in Table 2. In all these experiments a web of clot was found to form between the sting and the test chamber wall, apparently arising from a build-up of clot on the upstream edge of the furthest downstream portion of the sting, which was at right angles to the remainder, and hence perpendicular to the flow. A thin uniform layer of clot (1-2mm

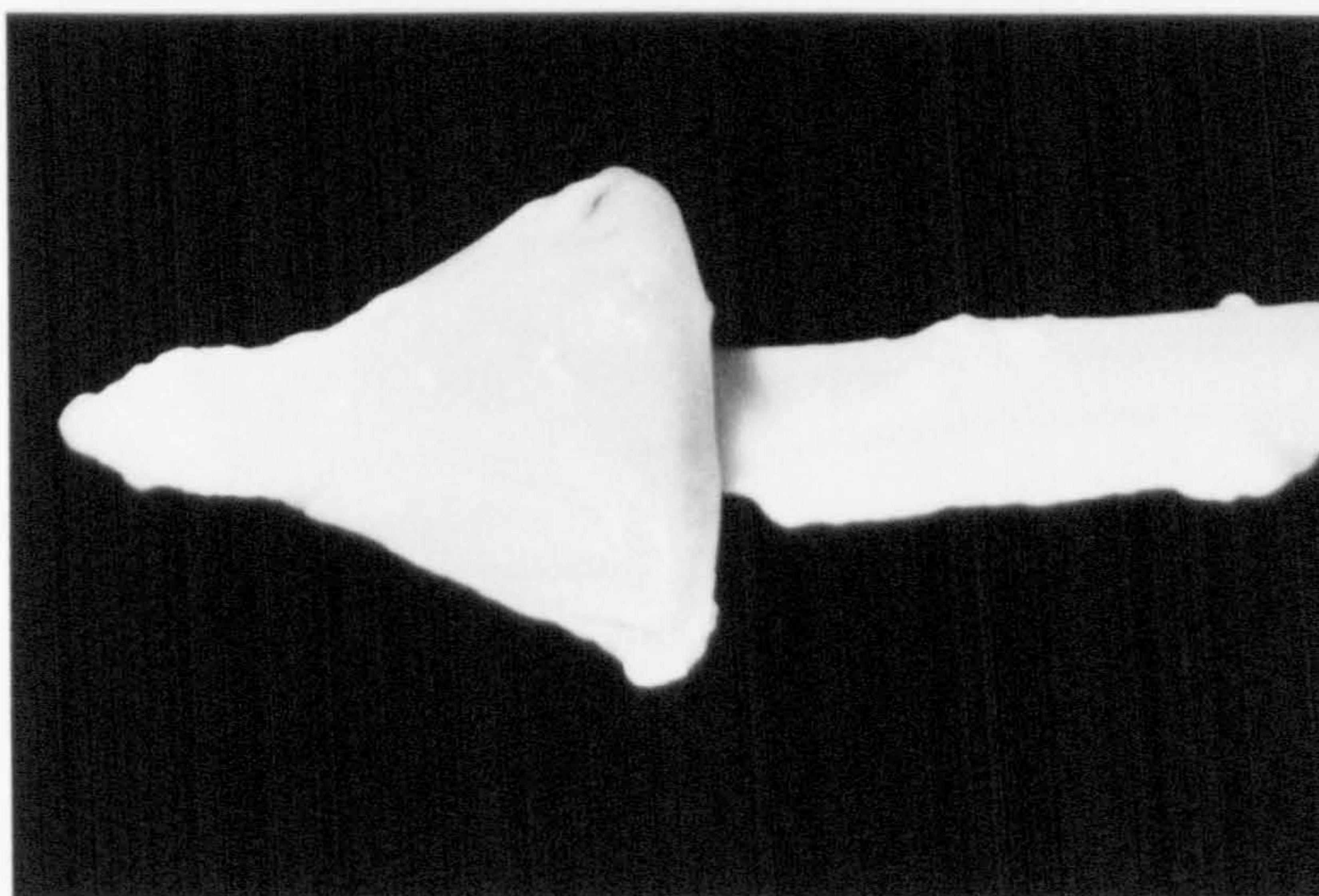




21a Tear-drop (pulsatile flow)



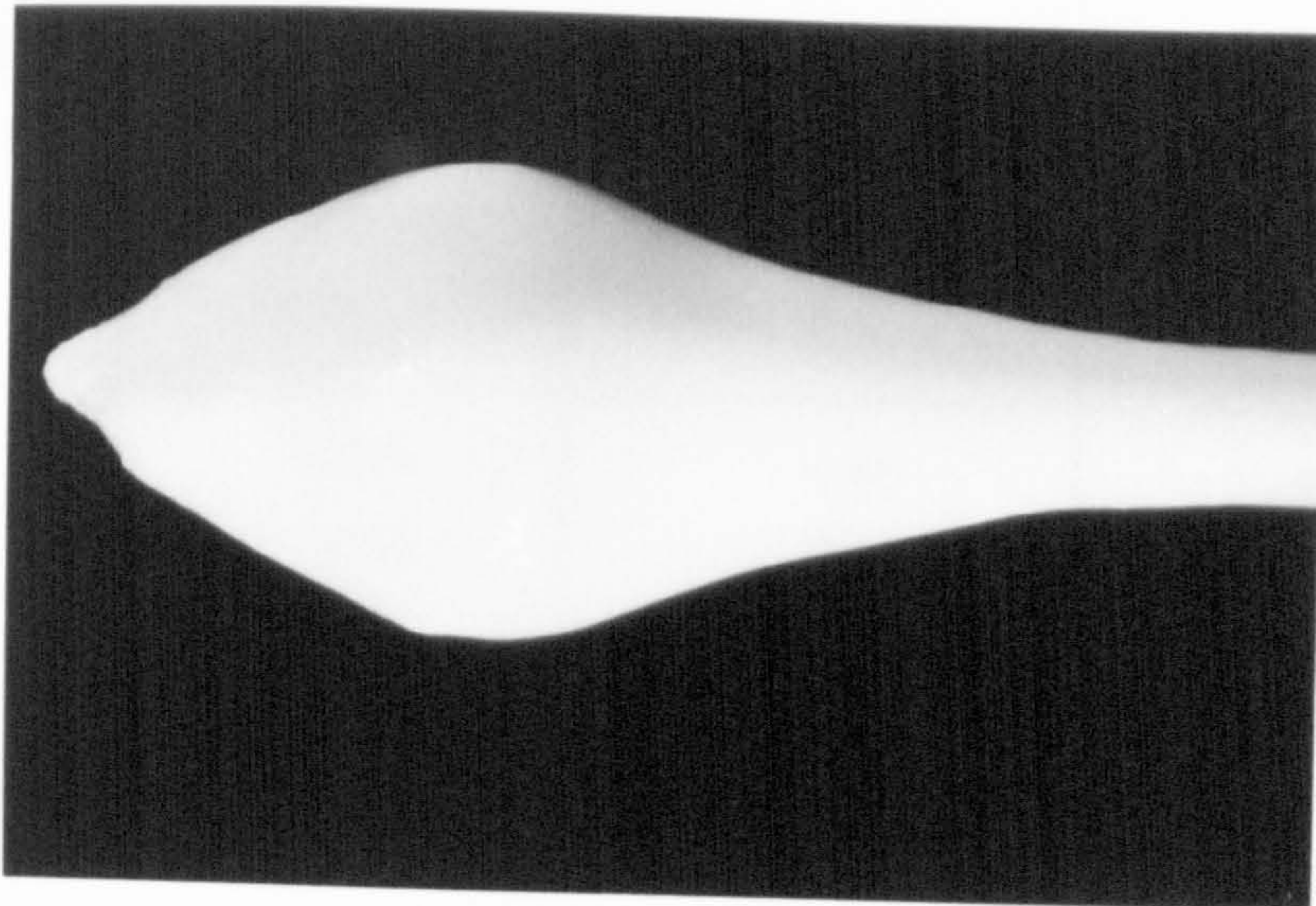
21b Sphere (pulsatile flow)



21c Upstream-Apex Cone (pulsatile flow)

Fig. 21 Photographs of Clot around Objects in the Long Chamber





21d Disc (pulsatile flow)

Fig. 21d Photograph of Clot around Disc in the Long Chamber



Disc (steady flow)

Fig. 22 Photograph of Clot around Disc in Steady Flow (Long Chamber)



**Table 2****Weight of Deposition around Objects in the Long Test Chamber**

Object	Wt. Upstream Clot / g	Wt. Downstream Clot / g	Wt. Total Clot/g
Disc	0.61	0.80	1.41
Tear-drop	0.05	0.49	0.53
Sphere	0.20	0.26	0.45
Up.-Apex Cone	0.18	0.11	0.28

thick) was observed on the test chamber walls. As this and other deposits were very fragile, it was essential that dismantling of the test chamber was carried out without any sudden movements, either of the test chamber, or of the residual milk. The objects in order of thrombus weight were upstream-apex cone, sphere, tear-drop and disc. The clot adhering to the object appeared to form a uniform covering except in the case of the cone and the disc. In the case of the cone, a long rod-like clot was observed, projecting from the apex along the cone axis; around the disc the milk curd formed a bi-conical shell, completely engulfing the test piece.

A second test was conducted with the disc in a 2l/min steady flow of milk. Again the disc was engulfed in approximately the same quantity (1.77g) of coagulum (Fig. 22), this time more loosely bound to it. The deposit on the upstream face was dome-shaped rather than conical, appearing similar to the expected build-up of particles obtained if such a disc were exposed to a flowing particle suspension, e.g. a snow-storm; there was fairly uniform deposition over the upstream face and a conical deposit downstream resulting from particles being swept into the wake.

In an attempt to determine whether this deposition was indeed a 'snowstorm effect', brought about by capture of preformed particles from a suspension, a third set of experiments was conducted. The disc was exposed to a pulsatile flow of milk in one test for one minute and in another for five minutes. In the former test specks of clot were observed to have impinged on the upstream face of the object, the downstream face of the disc remaining free of clot. In the latter the upstream layer of clot was almost 1mm deep (weighing 0.048g) and a 2mm layer was observed downstream (weighing 0.077g). The upstream and downstream surfaces of this coagulum appeared particulate in nature, thus suggesting that such deposition is indeed the result of preformed particles suspended in the flow impinging on the test-body.

The Lee White clotting time for the milk mixture used in these experiments is 30 seconds, so that the milk would be expected to clot shortly after the exit of the test chamber rather than within the chamber. However, the Lee White clotting time is a measure of the time taken for an immobile clot to form, fully occluding the test tube. Small particles of coagulating protein are possibly present before this time. It is also possible that flow disturbances due to the entrance region may accelerate the coagulation reaction by aiding diffusion; this, however, appears unlikely in view of the experiments reported in Chapter 7. A chamber with no more than a diffuser was therefore adopted to eliminate this problem.

### 6.1.3 Short Test Chamber

The deposition of milk clot on the tear-drop from both steady and pulsatile flows of milk was observed in this chamber. Early tests using 15 gallons of milk proved difficult in that the milk clot appeared to be more loosely bound at this point. The milk temperature was raised to 41°C and the experimental duration extended by the use of 30 gallons of milk, so as to increase both the rate and duration of the fluid reaction, with the aim of improving the adhesion of the formed clot.

Both steady and pulsatile flow led to similar amounts of deposition, though the shape of the deposited clot was different in each case (Fig. 23, Table 3 ). In steady flow a smooth streamlined clot formed on the downstream side of the object, whereas in pulsatile flow a thicker band of clot gave an abrupt downstream end to the clot. The clot on the upstream face in each case appeared sponge-like and particulate in nature, giving the impression that it had been formed from a suspended precipitate in the fluid impinging on the surface. A further discussion of these experimental observations is included in the next subsection.

**Table 3**  
**Weight of Deposition around Tear-drop in the Short-Test Chamber**

Flow Regime	Wt. Upstream Clot /g	Wt. Downstream Clot /g	Wt. Total Clot/g
Pulsatile	0.087	0.63	0.72
Steady	0.096	0.49	0.59



This test involved 30 gallons of milk, increasing the length and reducing the number of experiments able to be performed in a limited time. A compromise between the long and short test sections was thus tested, having just under half the residence time, between injection and test object, of the long test section. It was hoped that 15 gallons of milk would suffice with this test section. This arrangement also allowed the flow to stabilise prior to encountering the test object.

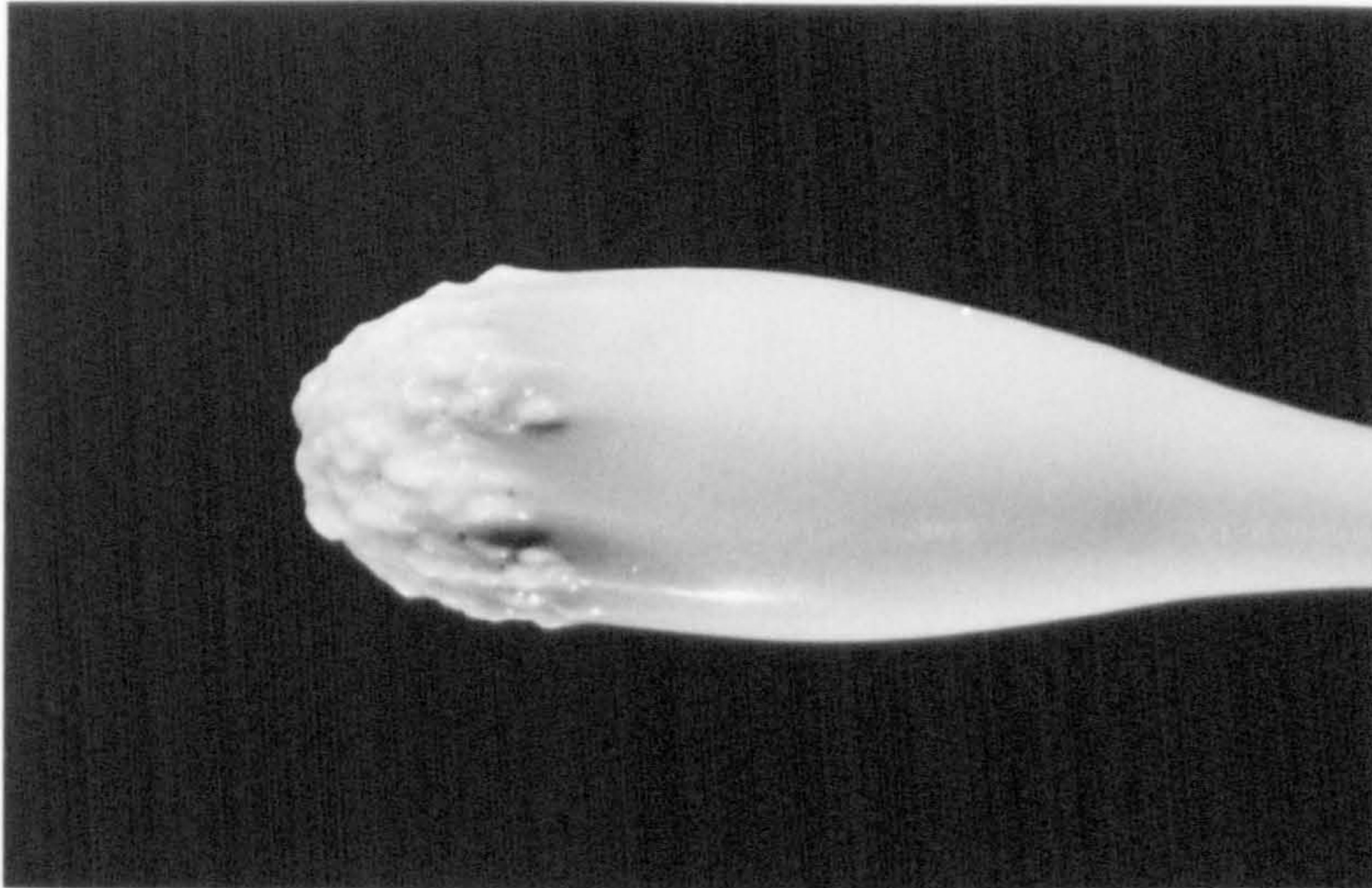
#### 6.1.4 Medium Length Test Chamber

In this test chamber the coagulation around the tear-drop, sphere, disc and upstream apex cone were compared for both steady and pulsatile 2l/min flows of milk. The downstream-apex cone was observed in pulsatile flow alone. The photographs of the resulting clots are shown in Figures 24 and 26 and the weights of clot recorded in Table 4.

Observations of the milk clot formed on the upstream face of the object (e.g. see Figs. 24 and 26 ) indicate that its nature may be different from that found in the 'wake' region. The upstream clot varies more in quantity from run to run, is generally more randomly spread and has a more coarse-grained spongy appearance than that found downstream. It is possible that this random deposit is adventitious, being caused by vapour bubbles adhering to the object, by surface irregularities, or by impinging particles of clot dislodged from the test chamber wall further upstream. In Figure 26c, for example, crater-like clots appear to have formed around bubbles of vapour on the surface. Attempts were made to eliminate all bubbles from the system prior to the experimental run but these were not always successful. The clot adhering to the upstream face of the tear-drop, especially in steady flow, appears to originate around scratches on the PVC surface - perhaps in a process similar to the formation of wedge-type clots in the stagnation point flow chamber experiment. Occasionally spongy spots of clot are found in regions where no surface imperfections are apparent. These may be the result of particles of clot, dislodged upstream from the test section wall, impinging on the face of the test-piece. Since the upstream coagulum is thus considered to arise for reasons not necessarily connected with the shape of the object, the weight of clot in the downstream region has been assessed independently, although both sets of weights are given for reference in Table 4.

The deposited coagulum in the wake region is generally smoother and more uniformly spread azimuthally around the object, indicating a more ordered deposition pattern. In steady flow the wake region downstream of all the objects is the site of a smooth cone shaped milk clot (Fig. 24 ). This conical clot appears to have formed preferentially to one side of the strut with the



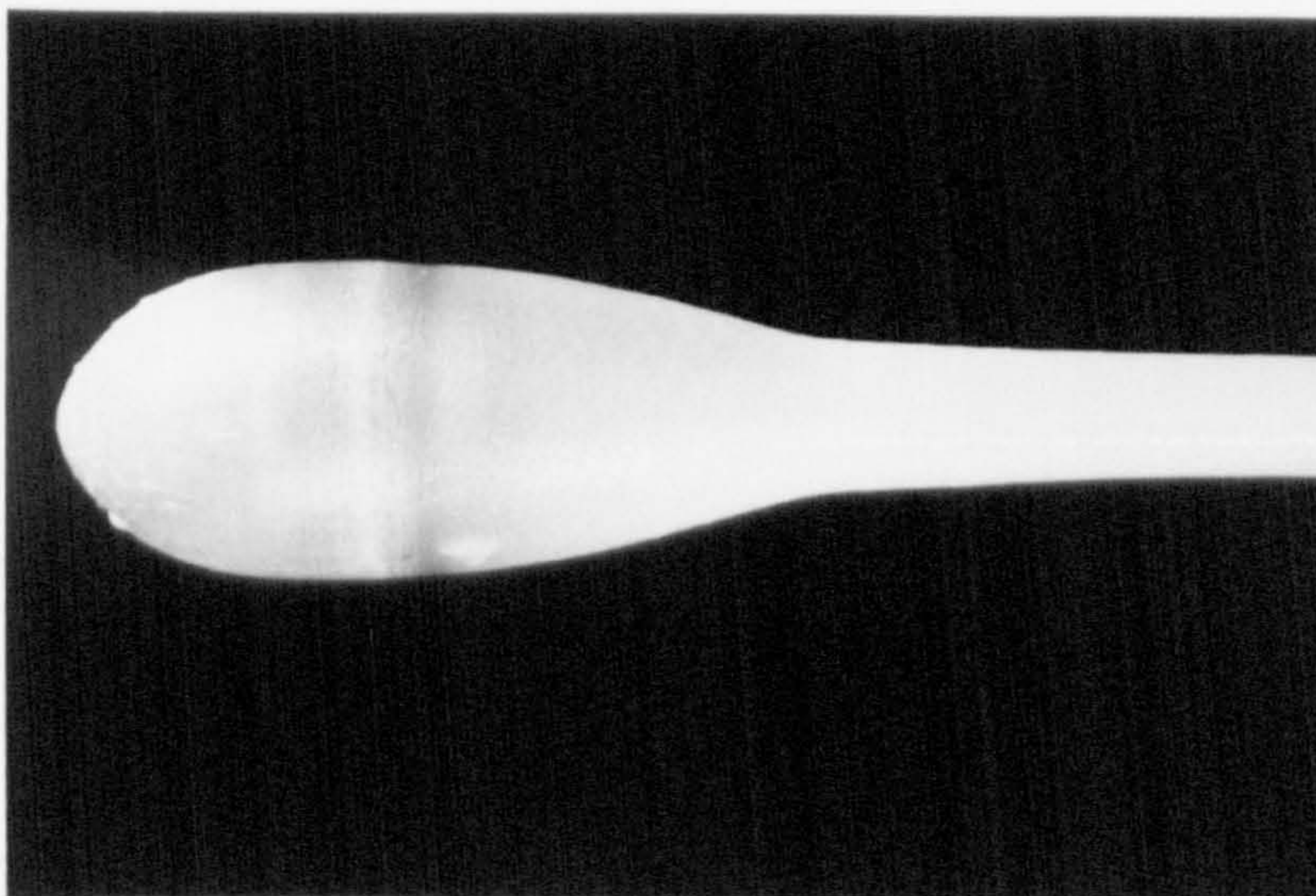


23a Steady Flow



23b Pulsatile flow

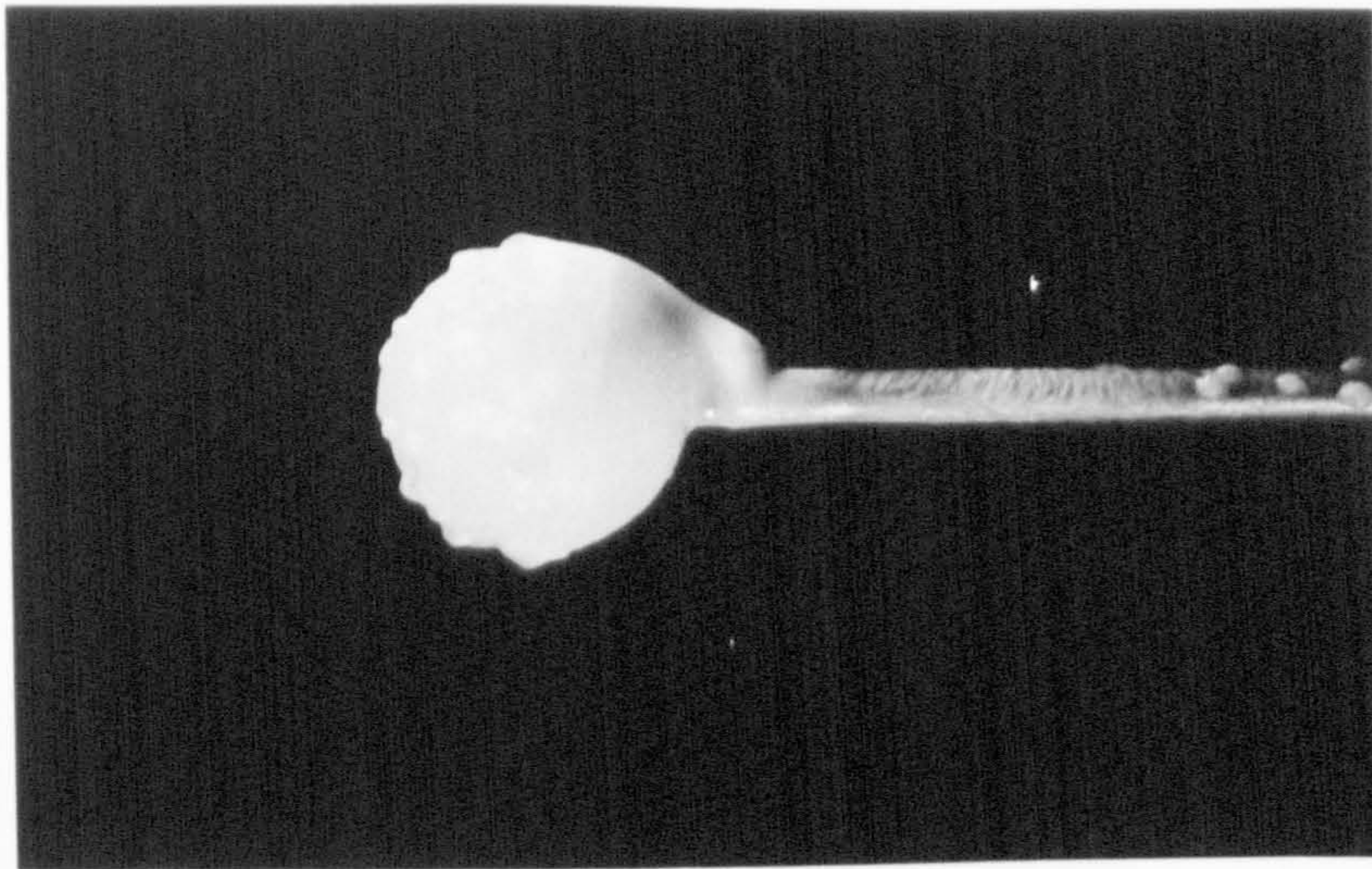
Fig. 23 Photographs of Clot around Tear-drop in the Short Chamber



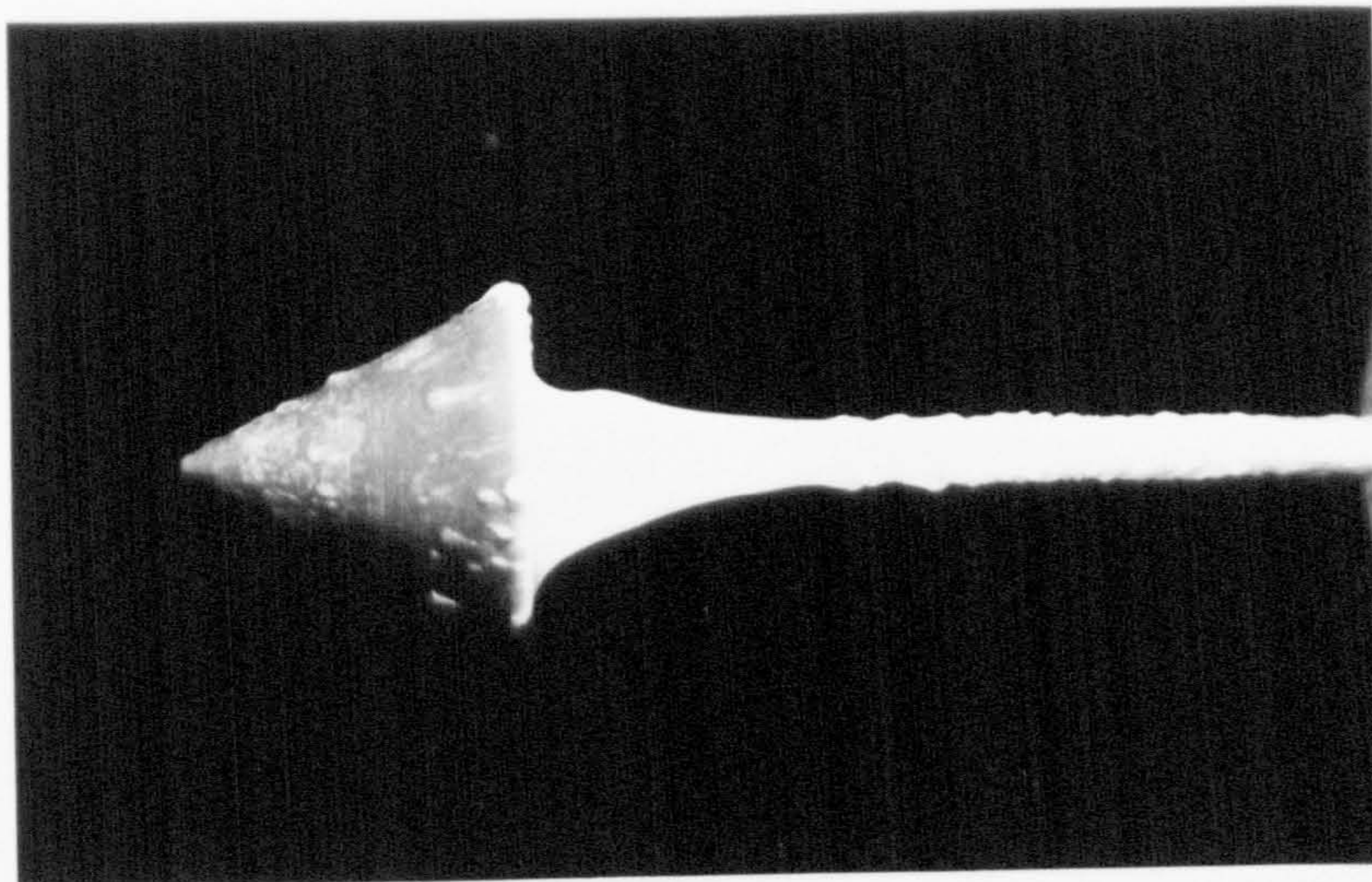
24a Tear-drop (steady flow)

Fig. 24a Photograph of Clot around Tear-drop in the Medium Chamber





24b Sphere (steady flow)



24c Upstream-Apex Cone (steady flow)



24d Disc (steady flow)

Fig. 24 Photographs of Clot around Objects in the Medium Chamber



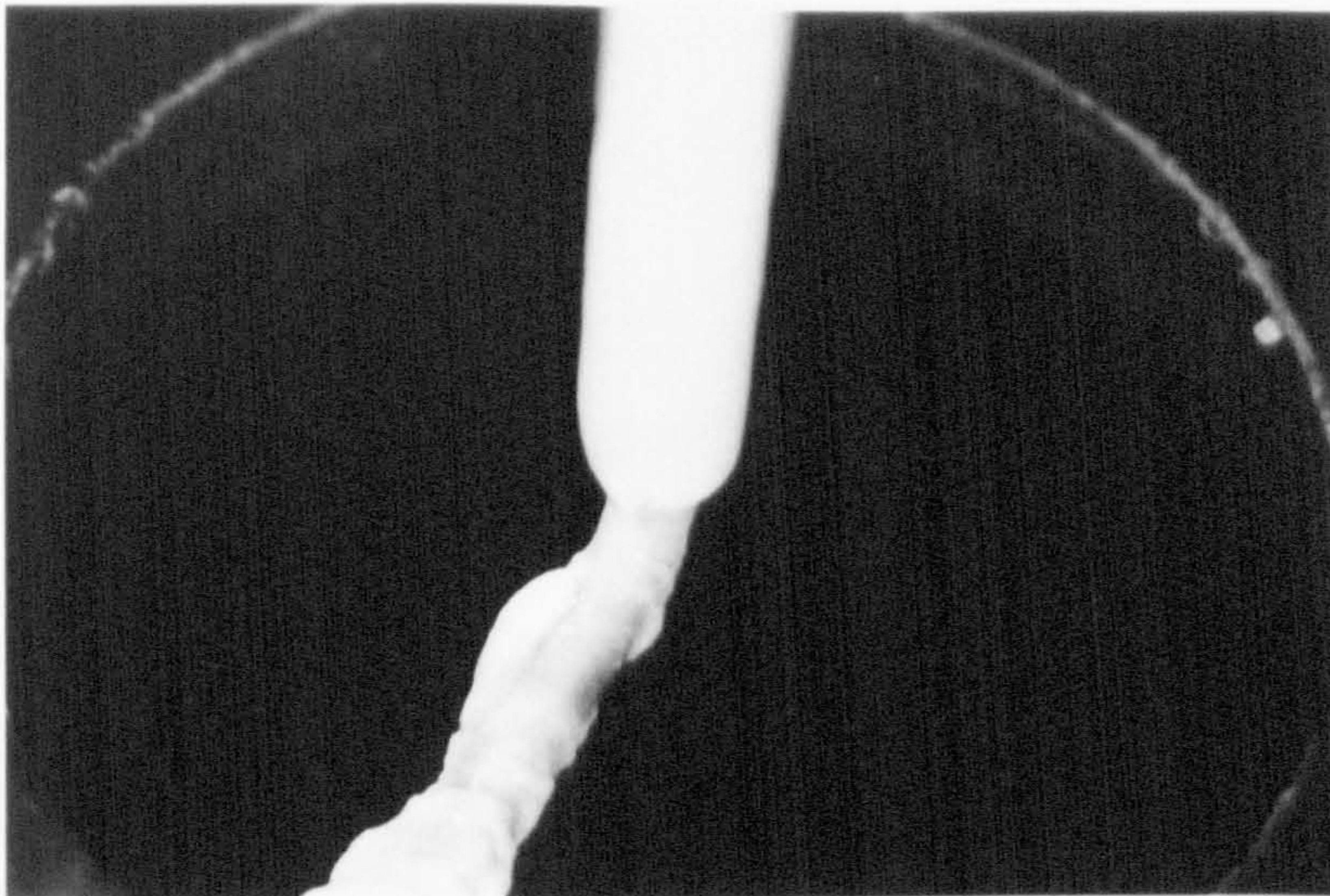
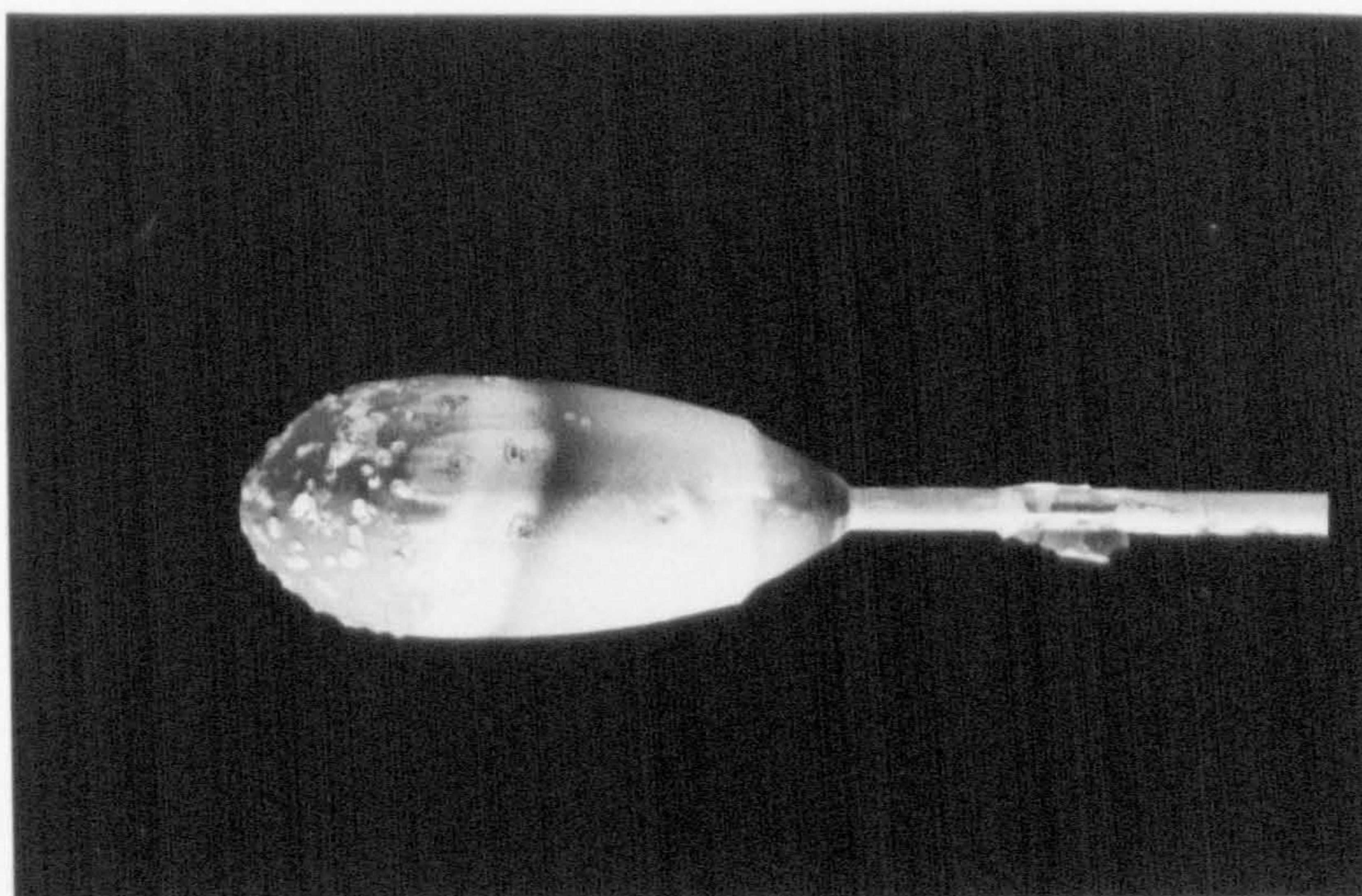
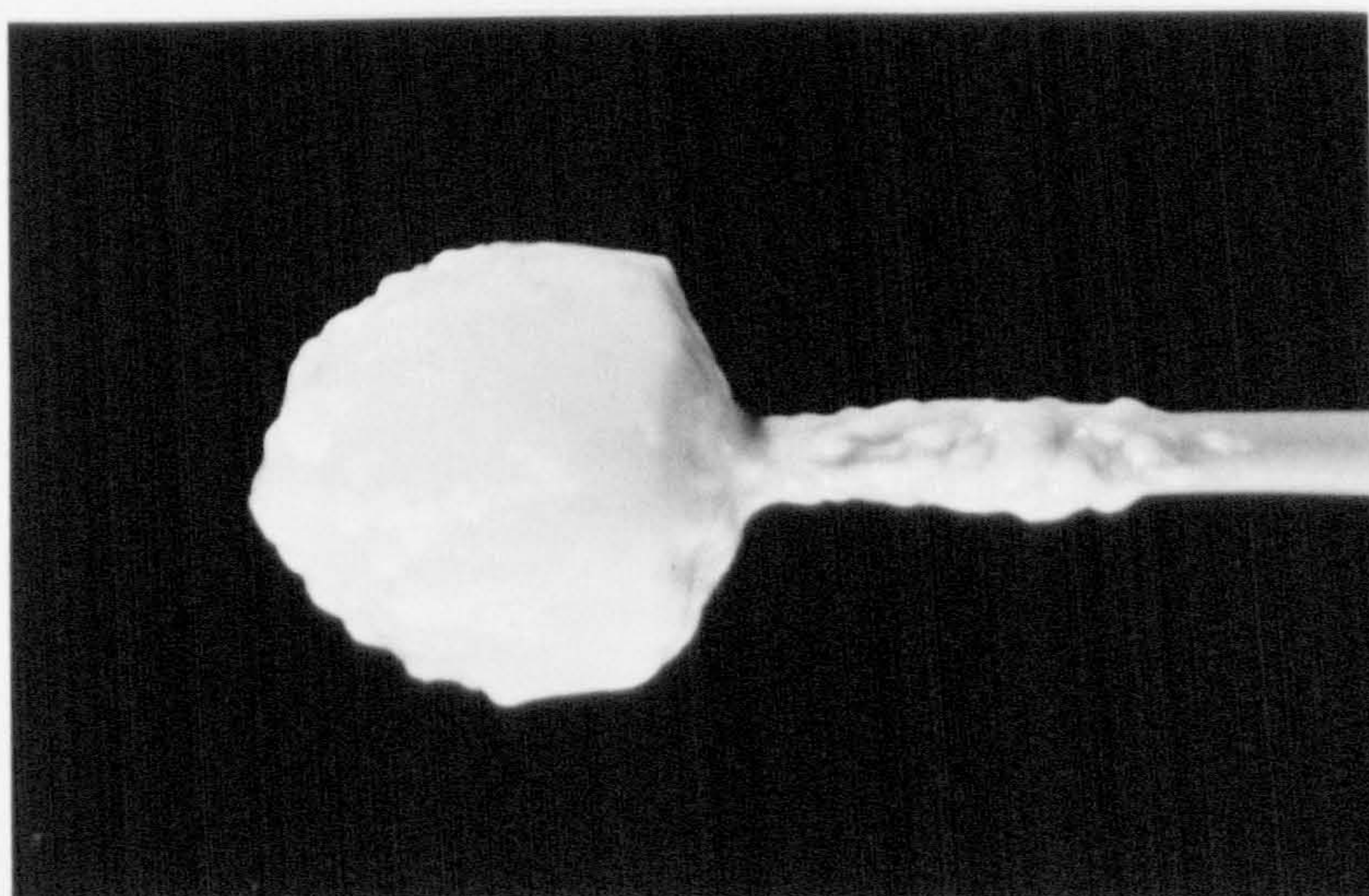


Fig. 25 Photograph of Clot on Sting with Tear-drop in Steady Flow



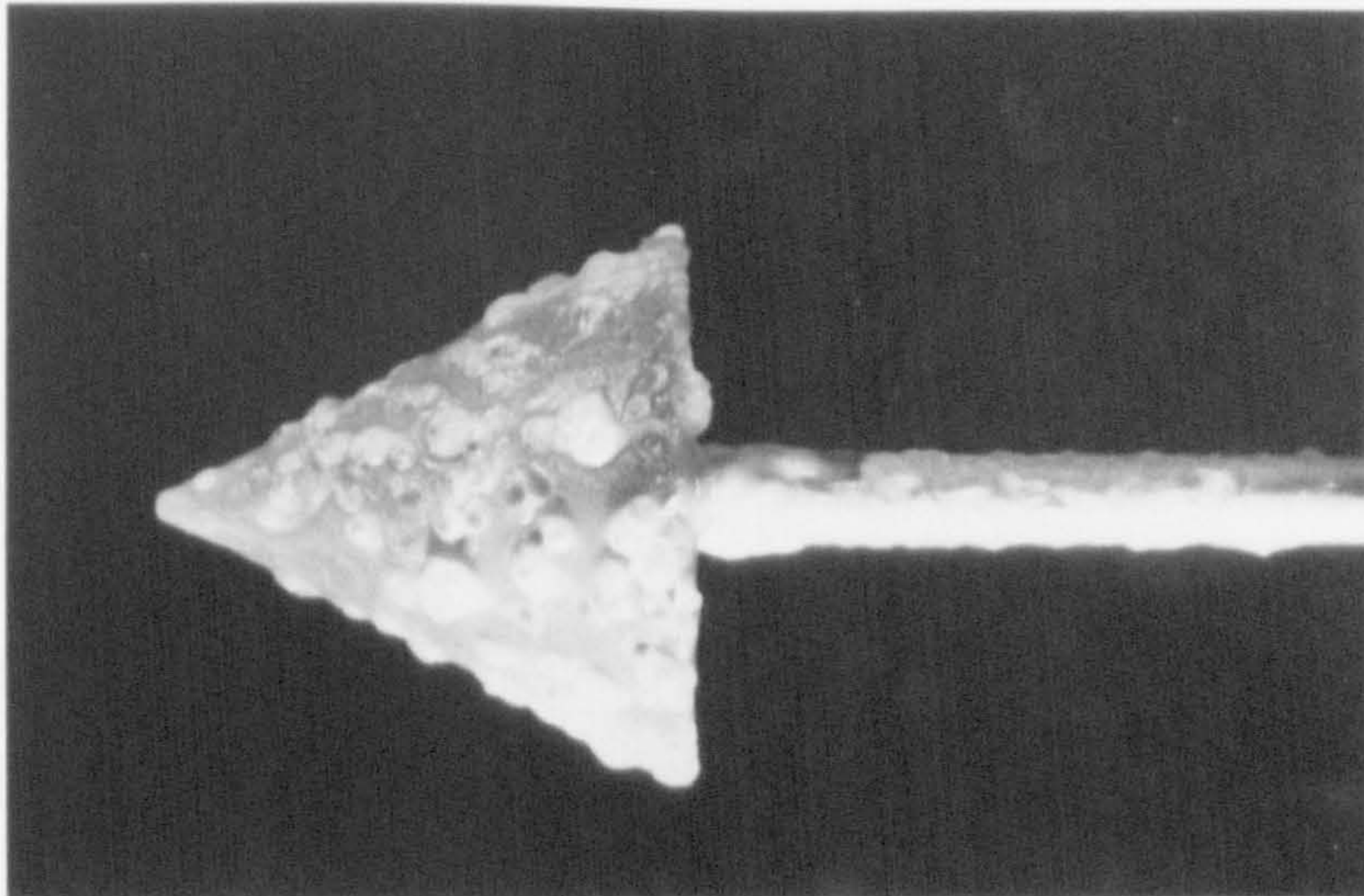
26a Tear-drop (pulsatile flow)



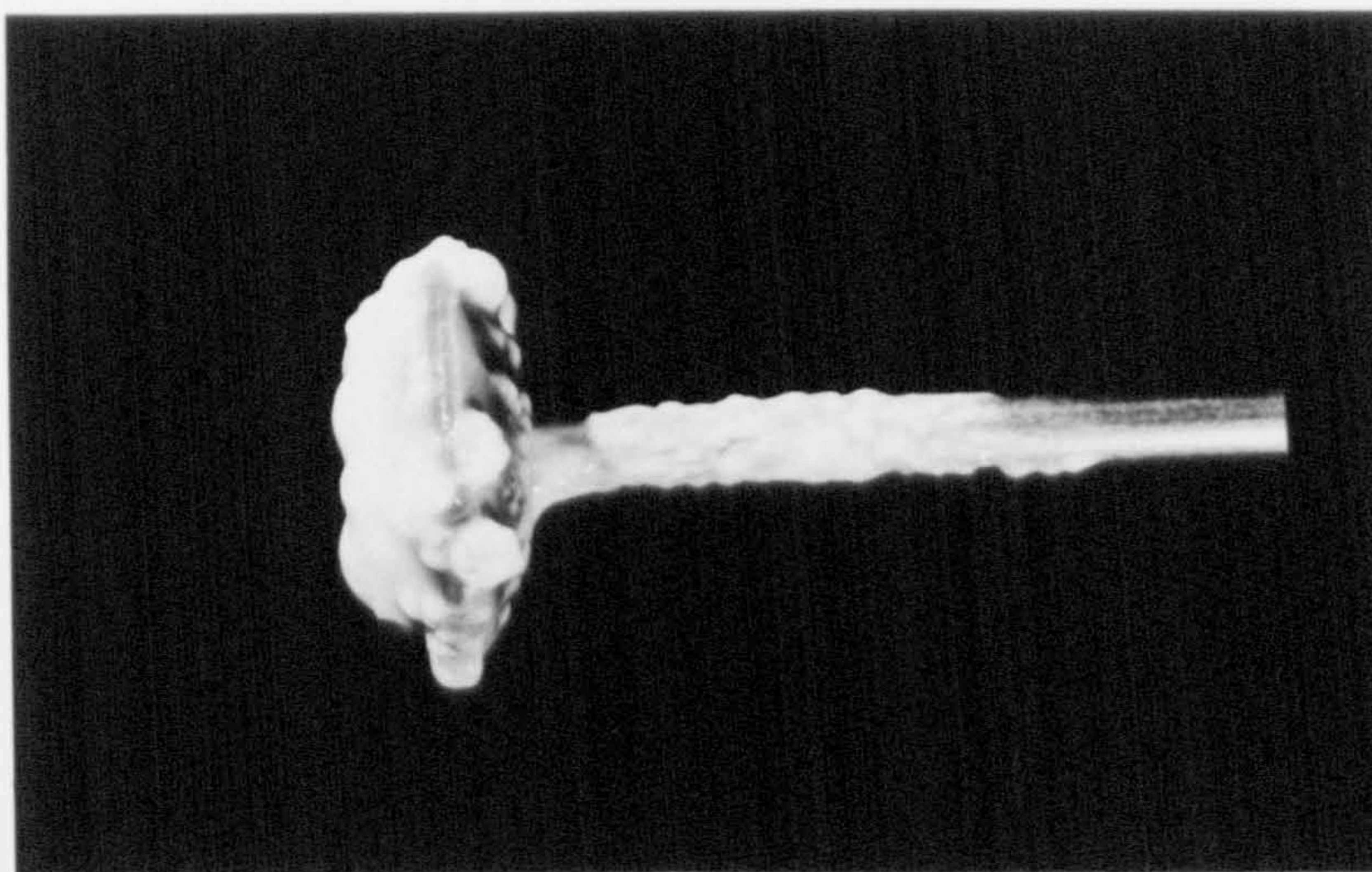
26b Sphere (pulsatile flow)

Fig. 26 Photographs of Clot around Objects in the Medium Chamber

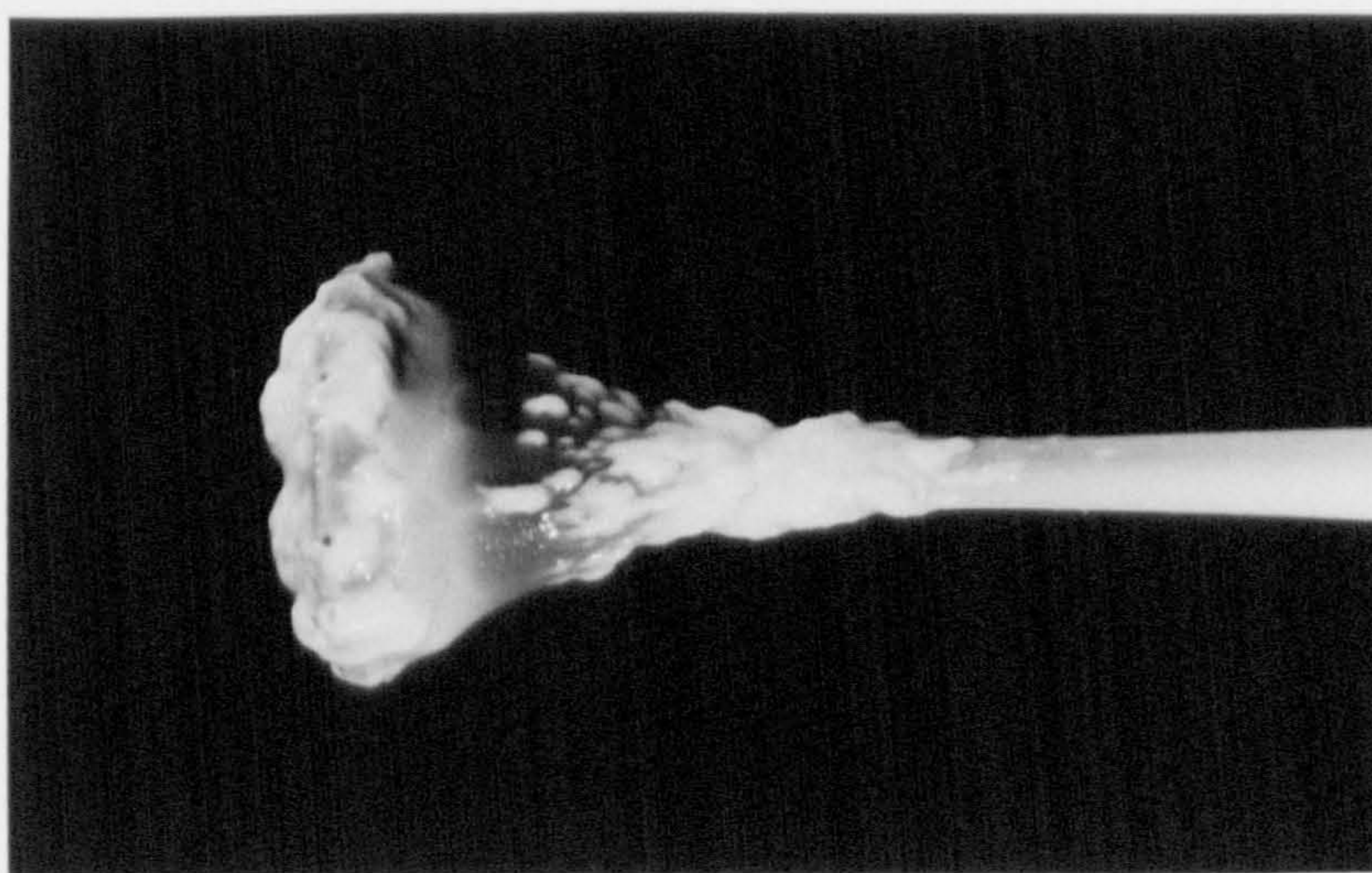




26c Upstream-Apex Cone (pulsatile flow)



26d Disc (pulsatile flow)



26e Downstream-Apex Cone (pulsatile flow)

Fig. 26 Photographs of Clot around Objects in the Medium Chamber



**Table 4****Weight of Deposition around Objects in the Medium Length Test Chamber****a) in Steady Flow**

Object	Wt. Upstream Clot / g	Wt. Downstream Clot / g	Wt. Total Clot/g
Disc	0.037	0.071	0.107
Tear-drop	0.004	0.080	0.084
Sphere	0.008	0.043	0.051
Up.-Apex Cone	0.012	0.095	0.107

**b) in Pulsatile Flow**

Object	Wt. Upstream Clot / g	Wt. Downstream Clot / g	Wt. Total Clot/g
Disc	0.026	0.027	0.053
Tear-drop	0.023	0.200	0.223
Tear-drop*	0.040	0.223	0.263
Sphere	0.059	0.073	0.132
Up.-Apex Cone	0.045	0.006	0.051
Down.-Apex Cone	0.037	0.074	0.111

\* The tear-drop was tested twice to ascertain whether the deposition pattern found is reproducible



disc, upstream-apex cone and sphere. In the case of the tear-drop this clot appears to have propagated along the length of the strut to form a smooth uniform coating (Fig. 25 ).

In pulsatile flow, on the other hand, the wake of the upstream-apex cone, and to a large extent the disc, is free from clotted milk, whereas a thick band of clot is observed 5/6 of the distance along both the tear-drop and the sphere (Fig. 26a & b ). This is the point where flow separation in forward flow might be expected to occur with each of these objects. In the case of the downstream-apex cone a sponge-like clot is observed on the upstream base of the cone as well as on the region near the apex. Smooth clot is only observed in a band immediately downstream of the base of the cone.

Whilst the location of milk curd deposition in steady flow was in agreement with previously held views concerning the importance of stagnation and stasis in regions of clot deposition (both in blood and milk)<sup>68,72,73,80</sup>, the presence of the band of curd on the tear-drop and the absence of clot downstream of the cone in pulsatile flow appeared anomalous.

Flow separation from, and reattachment to, the surface and occurrence of stasis were considered as possible causes for this unexpected deposition pattern. The position of the band of coagulum at the rear of the tear-drop coincides with the region where flow separation from the surface might occur in forward flow, but no such band of clot is observed at the trailing edge of the cone where flow separation also occurs. Reattachment of the flow for both test objects occurs on the supporting strut. In Figure 24 there is no evidence of any thickening of clot on the strut downstream of the object, though in Figure 23, using milk at 41°C with the tear-drop at the exit of the diffuser, a lumpy band of clot occurs where reattachment of the forward flow is expected. Thus the effects of flow reattachment are by no means clear. The presence of clot occupying a large proportion of the wake of all these axisymmetric test-bodies in steady flow suggests that areas of stasis, where fluid remains for periods longer than the mean residence time of the bulk flow, are favoured sites of clot deposition. It is not clear, however, whether flow separation, or stasis, is responsible for this clotting pattern. To proceed further, a knowledge of the residence time in the vicinity of test objects in both steady and pulsatile flow was required to ascertain whether this conclusion could account for the corresponding deposition patterns.

The weights of thrombus, reported by Vorhauer, adhering to the tear-drop and the cone were 0.0301g and 0.1478g respectively, leading to the suggestion that the intensity of disturbance in the wake may be responsible for the extent of deposition<sup>68</sup>. In the milk clotting experiments, the weight of clot recorded in steady flow for the cone exceeded that for the tear-drop, though not



significantly (Cone, 0.095g; Tear-drop, 0.080g ), whilst in pulsatile flow a 0.200g milk clot is deposited behind the tear-drop and only a negligible amount downstream of the cone (0.006g). Neither of these sets of data are in full agreement with that of Vorhauer.

Several differences exist, however, between the flow situations in these milk experiments and in the *in vivo* tests of Vorhauer, one of which in particular may account for this discrepancy. When designing our test chamber some difficulty was encountered in obtaining Vorhauer and Tarnay's paper<sup>68</sup>. The objects were therefore fabricated according to the sketch transcribed from their paper by Casci et al<sup>1</sup>. Our flow chamber was designed so that the objects occupy only one tenth of the flow area, thus reducing 'wall-effects' on the wake of the test object. It transpired, however, that the cross-sectional area of each of Vorhauer's objects was between a third and half that of the canine descending aorta; the flow would consequently be strongly influenced by the presence of the (compliant) blood vessel wall. For instance, Vorhauer's objects will experience a greater shearing force in the region of greatest radius than in our experiments, so deposition there will be much less likely.

In Vorhauer's tests the blood flow would lie between the extremes of the pulsatile and steady flow reported here. Pulsatile flow in our milk test rig is similar to the pulsatile flow encountered at the exit of the heart, with a stagnant diastolic period. The flow in the descending aorta is closer to steady flow with a superimposed pulsatile element, because of the elasticity of the aortic wall. The high pressure drop across the object will serve to enhance the steady aspect of the flow. Thus, Vorhauer's results are best compared with the steady flow results reported here, where indeed a qualitative similarity exists, with clot forming preferentially in the wake of the objects.

## 6.2 Bjork-Shiley Valve

Five experiments were conducted with the Bjork-Shiley valve, supported by a silicone rubber disc, in the long test chamber. In the first of these, under the same pulsatile flow conditions as those using the axisymmetric test bodies, a ramp of clot was found on the upstream side of the disc (Fig. 27 ), blocking the minor orifice, with a similar plug on the downstream side of the orifice. The upstream clot appeared to be anchored to the strut rather than the disc. The downstream clot had a central ridge, aligned with the flow, with hollows on either side before thickening again at the wall.

In the other four experiments, conducted by two undergraduate students, J. Beare and R. Paterson, a study was conducted to deduce whether the nature of clot deposition in the region of the valve in pulsatile flow is indicative of a preferential deposition during systole. The pulsatile flow experiments were



carried out using a flowrate of 1.5l/min and a frequency of  $70\text{min}^{-1}$ . Again, a ramp of clot (not shown in the photographs - Fig. 28 ) was observed to the upstream of the minor orifice. A greater quantity of clot than before was deposited (8g as opposed to 5g), however, on the downstream side of the disc, with no central ridge being apparent. The increase in quantity of deposition was possibly due to the increased residence time upstream of the valve at the lower flowrate (28 seconds rather than 21 seconds). The total volume of milk used was only two thirds of that at the higher flowrate, but the increase in particle concentration after the extra lag time would apparently outweigh the slightly shorter exposure time.

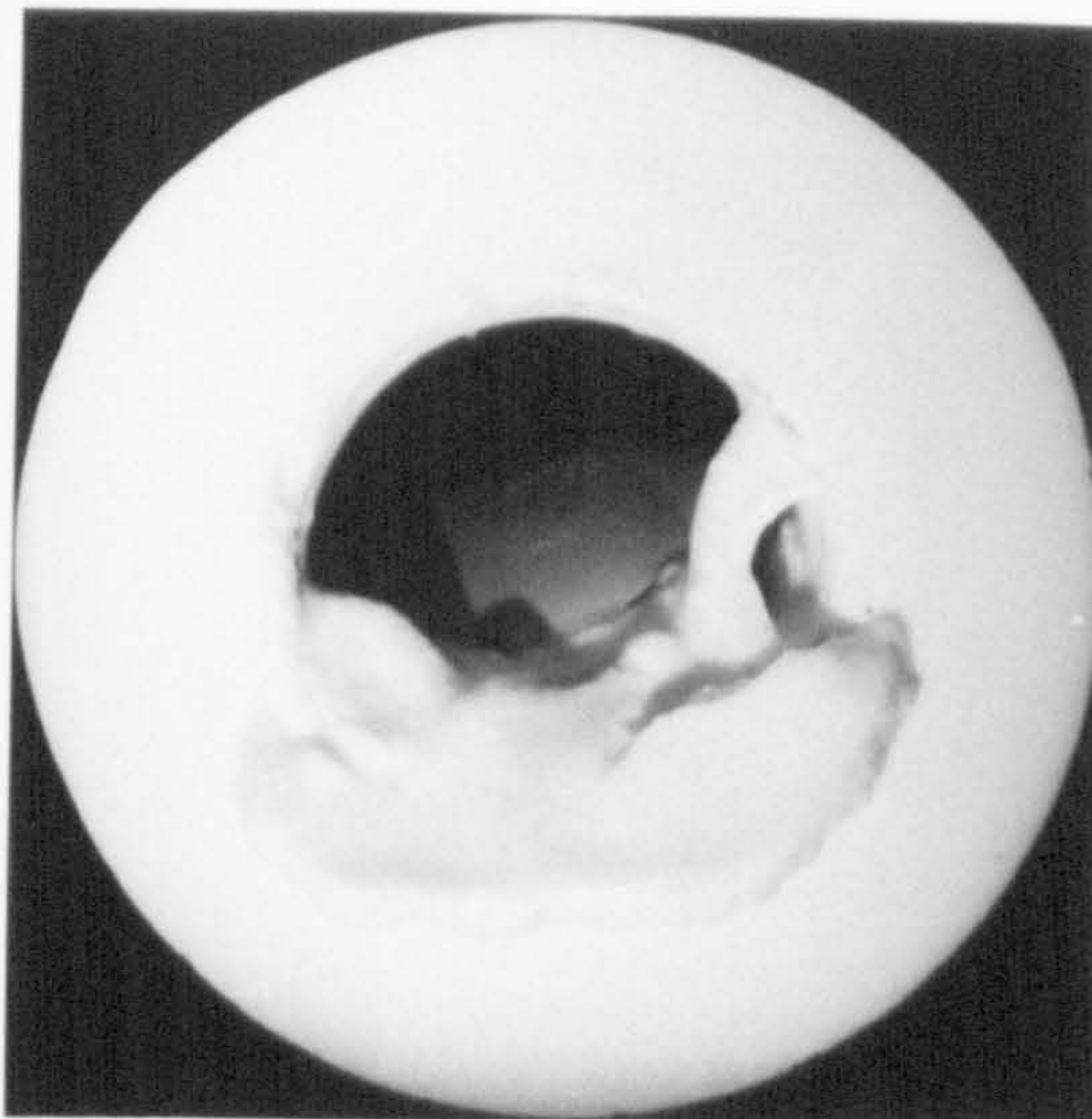
A flowrate of 3l/min was chosen for the steady flow experiments, to represent the average velocity during systole (systole accounts for half of the pulsatile period in these experiments). In one of these experiments the shape of clot deposited was similar to that in pulsatile flow (Fig. 29a ), with the clot being slightly more adherent to the surface of the disc. This is in contrast to other steady flow experiments where the clot formed on the walls of the test chamber and on the test objects appeared to be of a looser structure with less adhesion than in pulsatile flow.

In the second steady flow experiment, the rennet injection line developed a leak. An attempt was made to rectify the situation but this was not totally successful, so that the rennet injection flowrate was less than normal. This lower rennet concentration would have resulted in an increased Lee White clotting time. The duration of the experiment was also reduced. As expected, much less coagulum was deposited; a thin layer of clot covered the downstream surface of the disc (Fig. 29b ), whilst only a cluster of clot particles were found on the upstream face. The base of the upstream strut was the site of a slightly larger deposit.

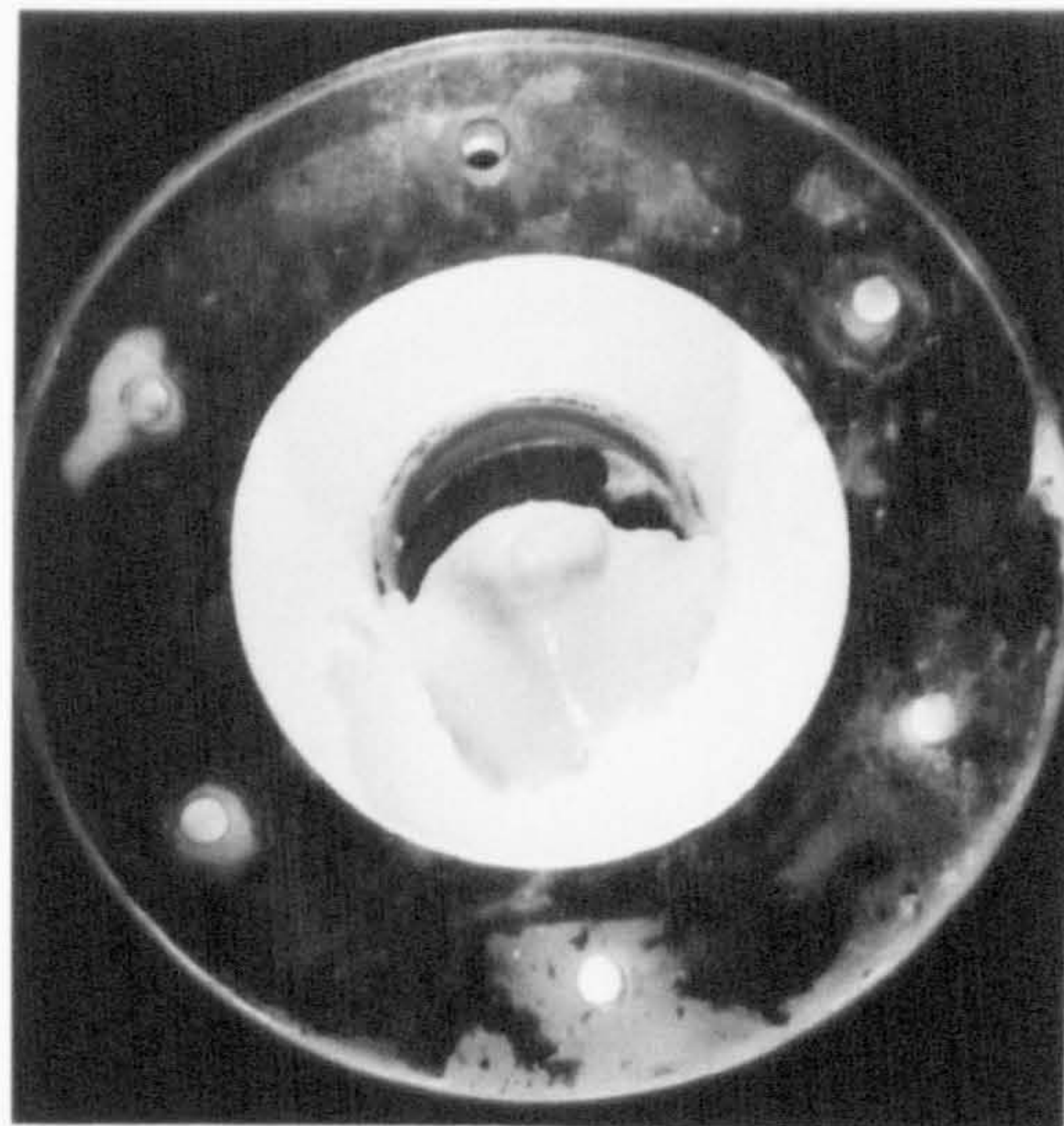
These experiments were carried out with the long test chamber. Therefore the above analysis of the deposition around axisymmetric objects in the long test chamber is likely to apply here; the mean residence time of the milk encountering the valve is sufficiently close to the Lee-White time to allow small particles of clot already to have formed in the slower-moving regions of the fluid stream. Thus the build-up of a ramp of clot in steady and pulsatile flow, both upstream and downstream of the minor orifice, may be due to the collection there of particles carried along in the fluid stream - a sedimentation process.

That the layer of clot in the second steady flow experiment was so thin is unlikely to be due to the early termination of the test run, as the run was only shortened by 30%. A more plausible explanation is that the loss of rennet led



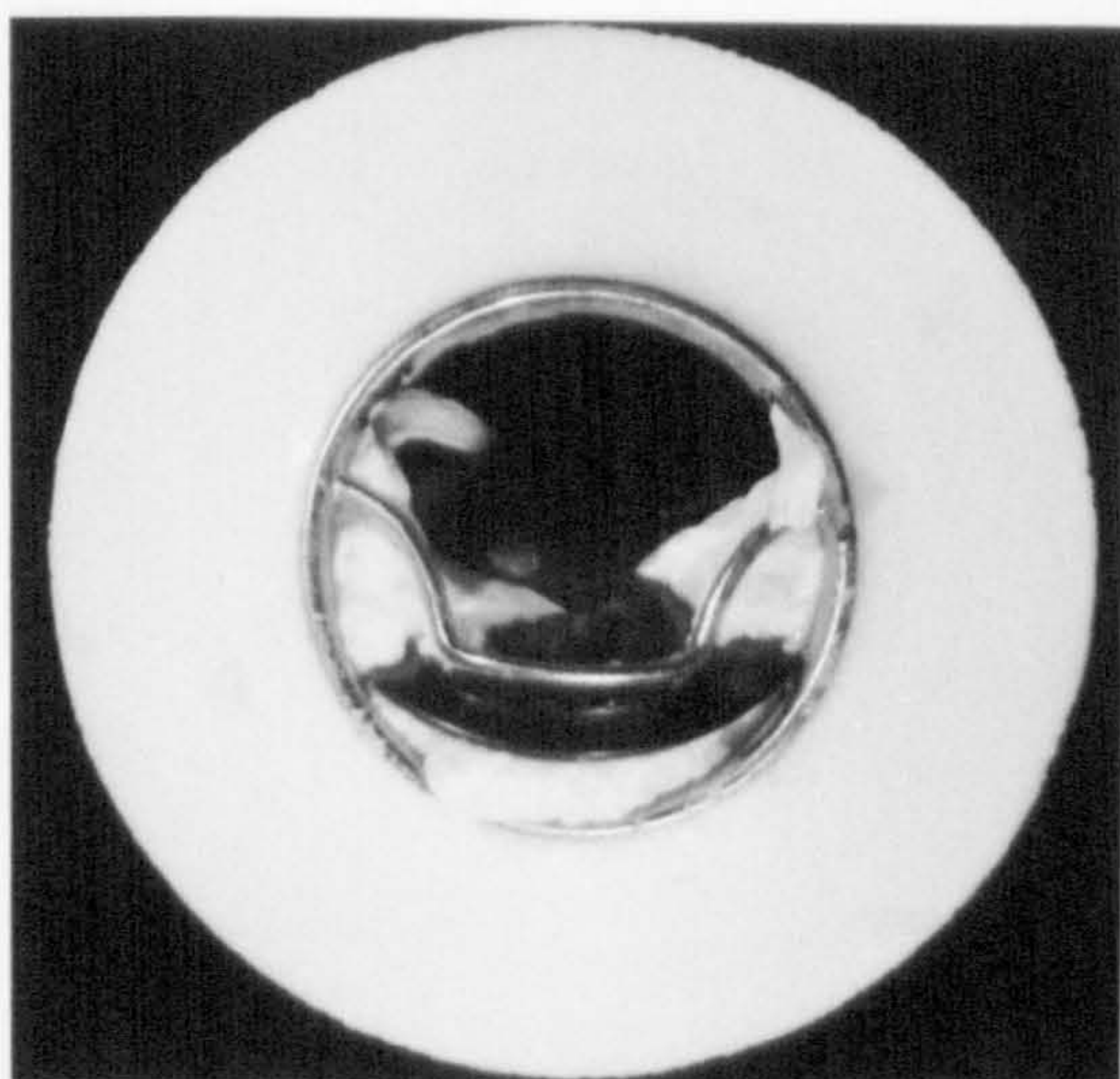


Upstream View



Downstream View

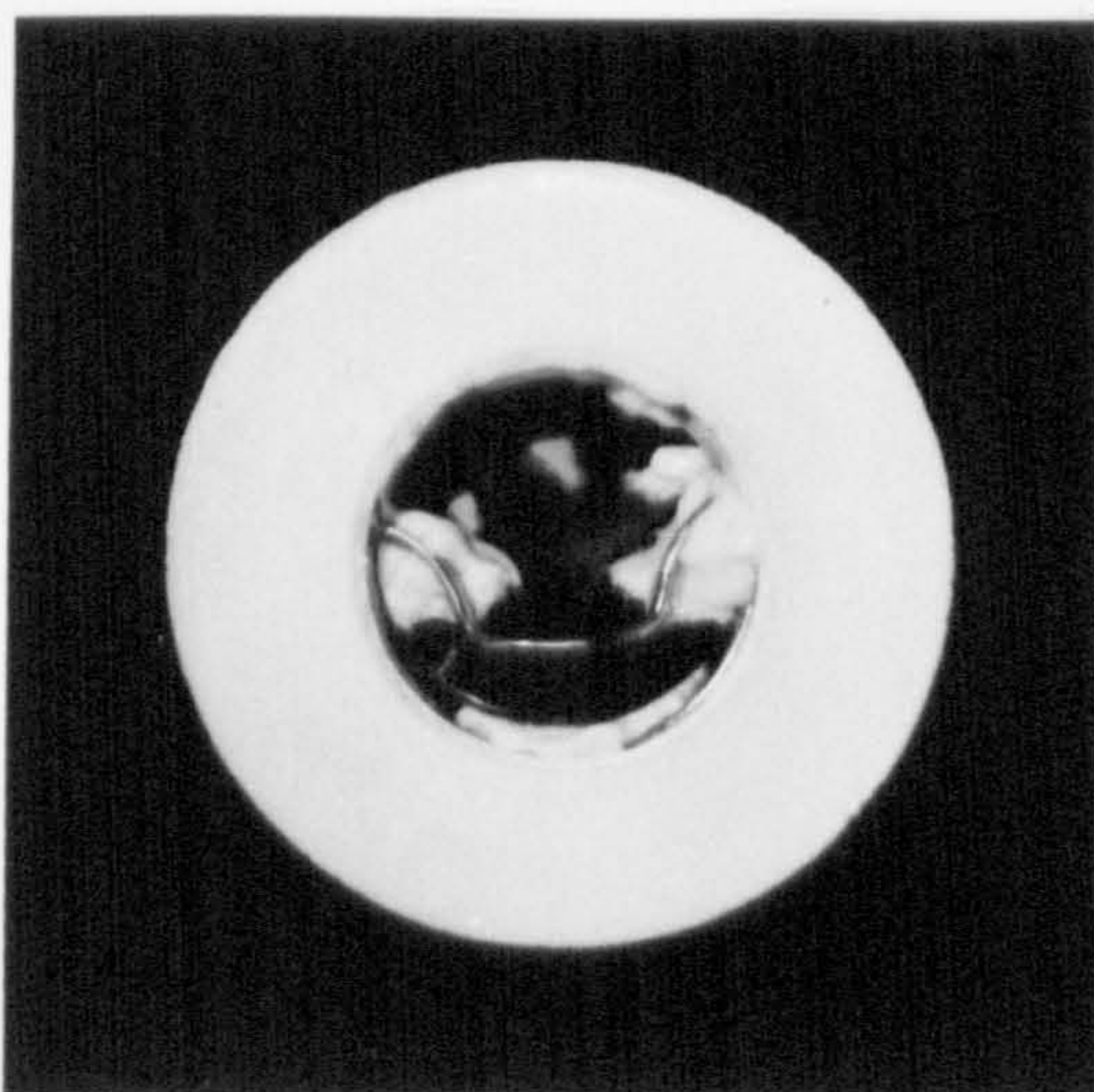
Fig. 27 Photographs of Clot around Bjork-Shiley Valve in 2l/min Pulsatile Flow



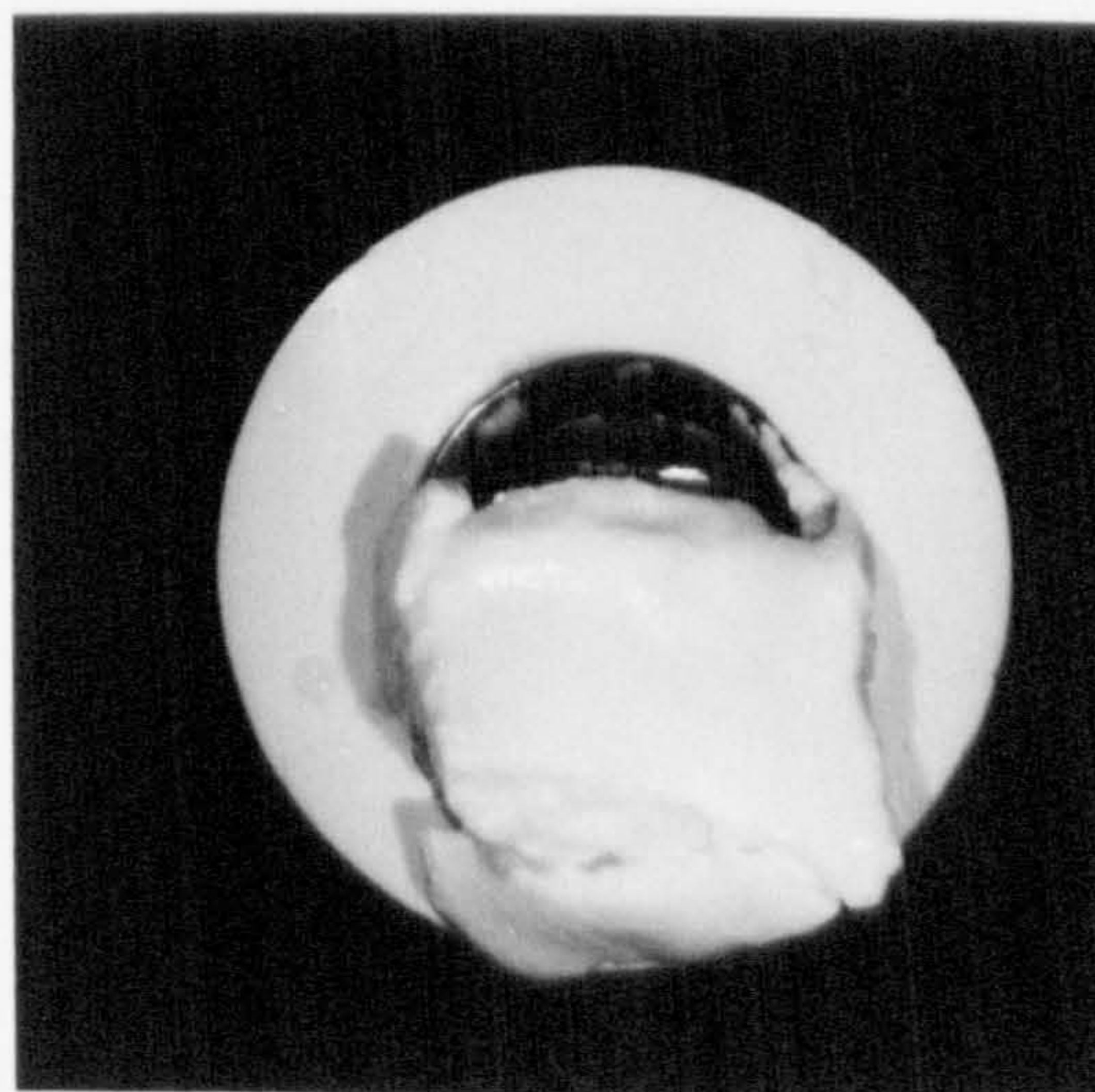
Upstream View (Run 1)



Downstream View (Run 1)



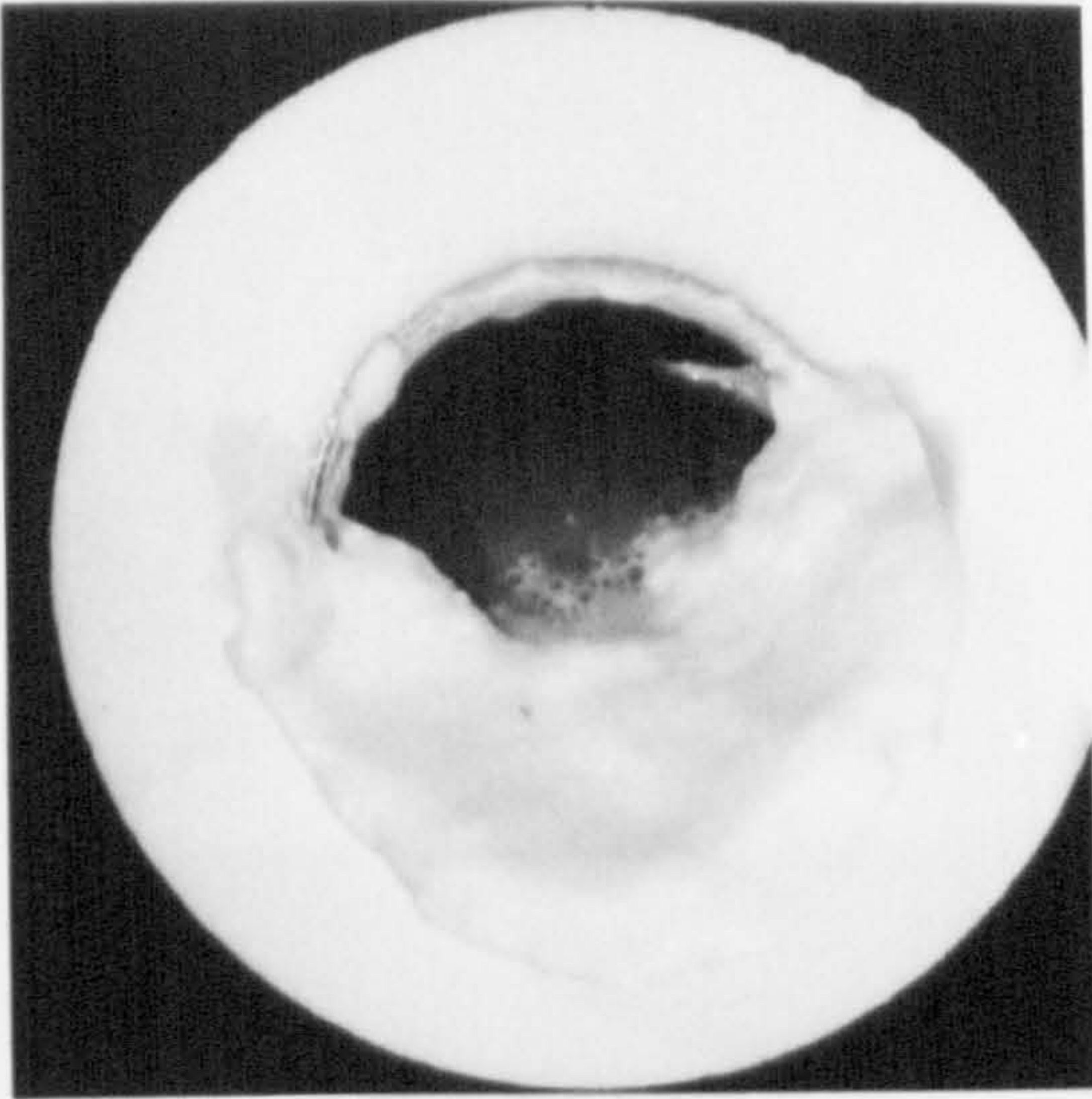
Upstream View (Run 2)



Downstream View (Run 2)

Fig. 28 Photographs of Clot around Bjork-Shiley Valve in 1.5l/min Pulsatile Flow

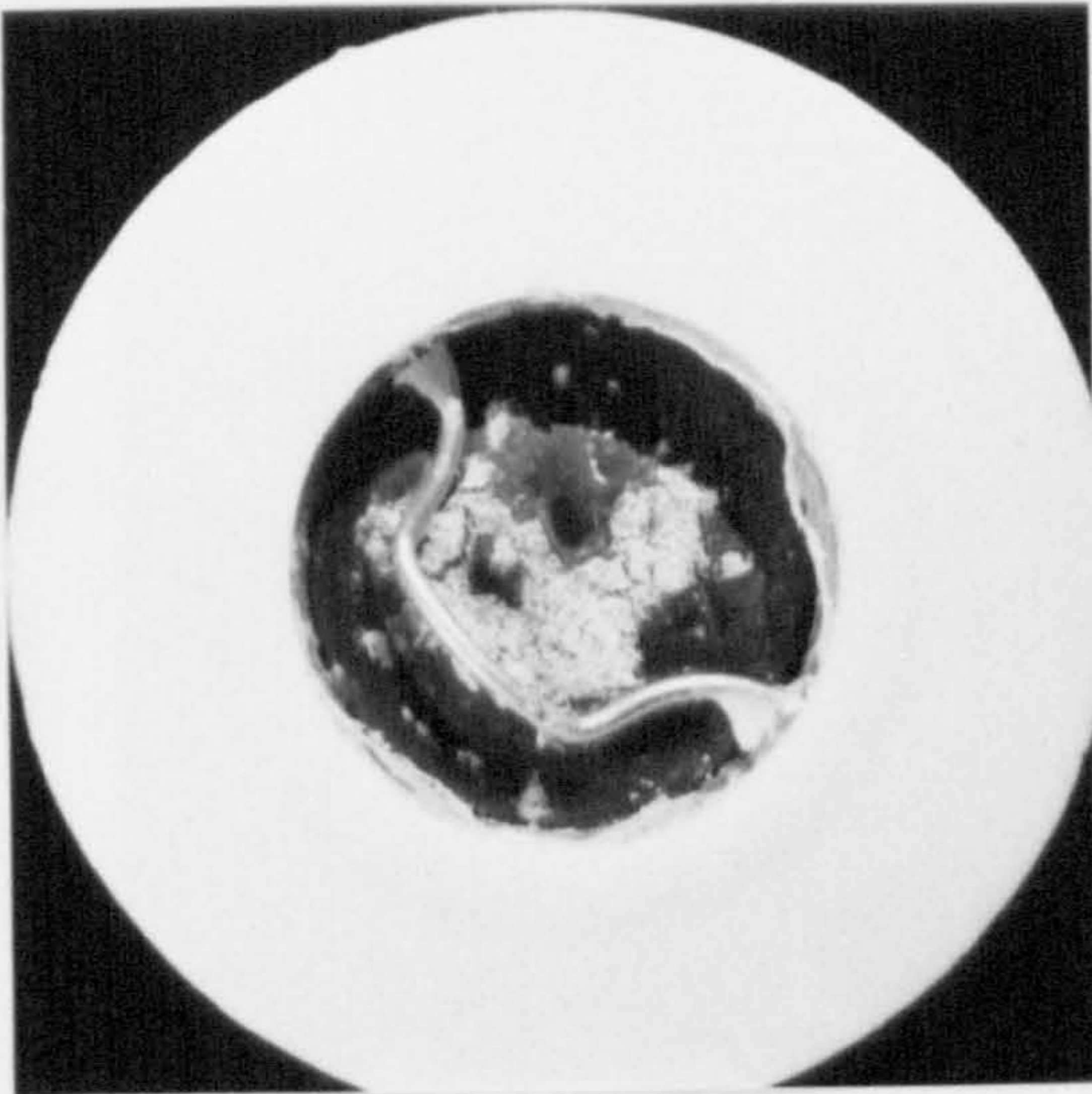




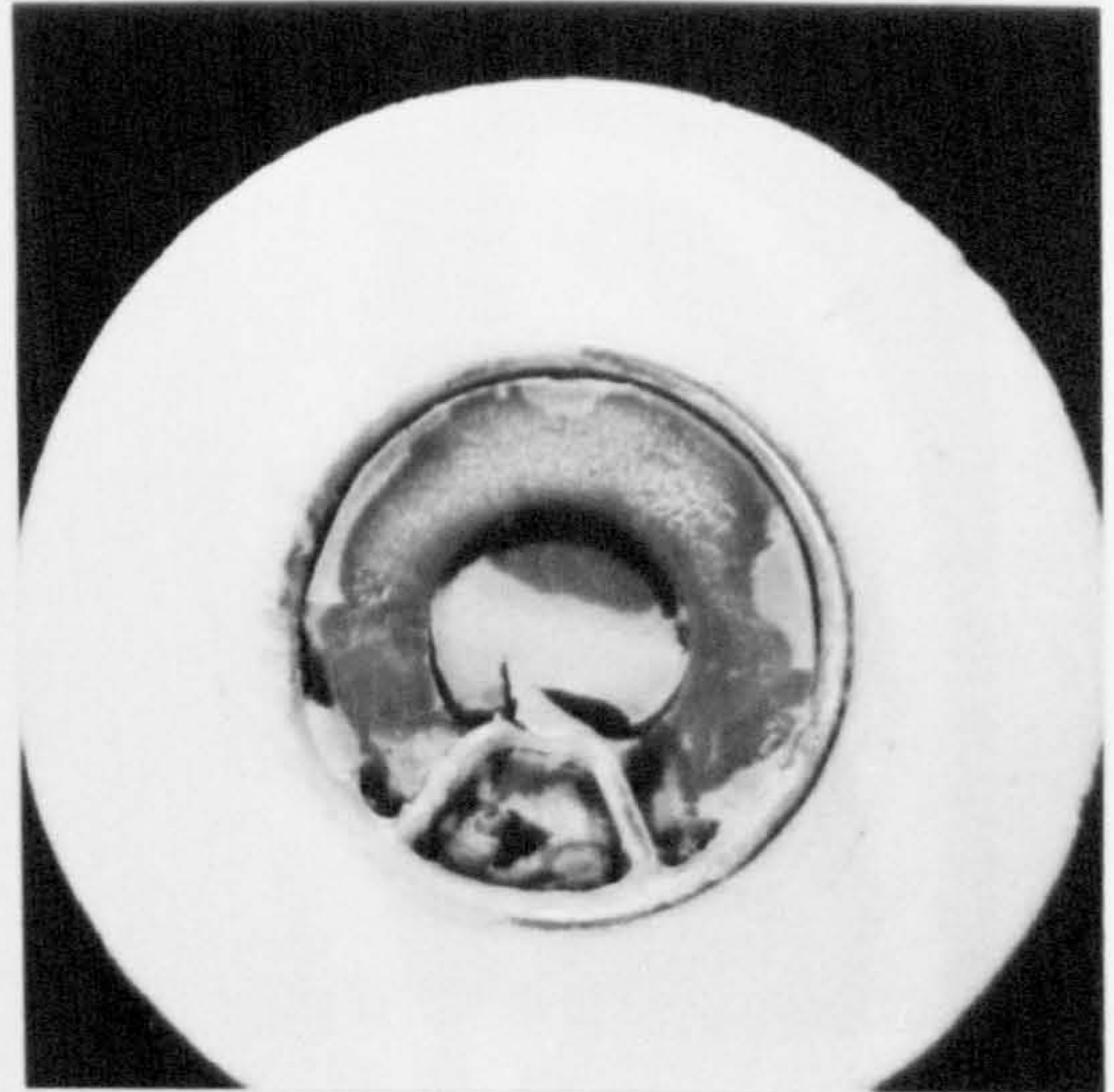
Upstream View (Run 1)



Downstream View (Run 1)

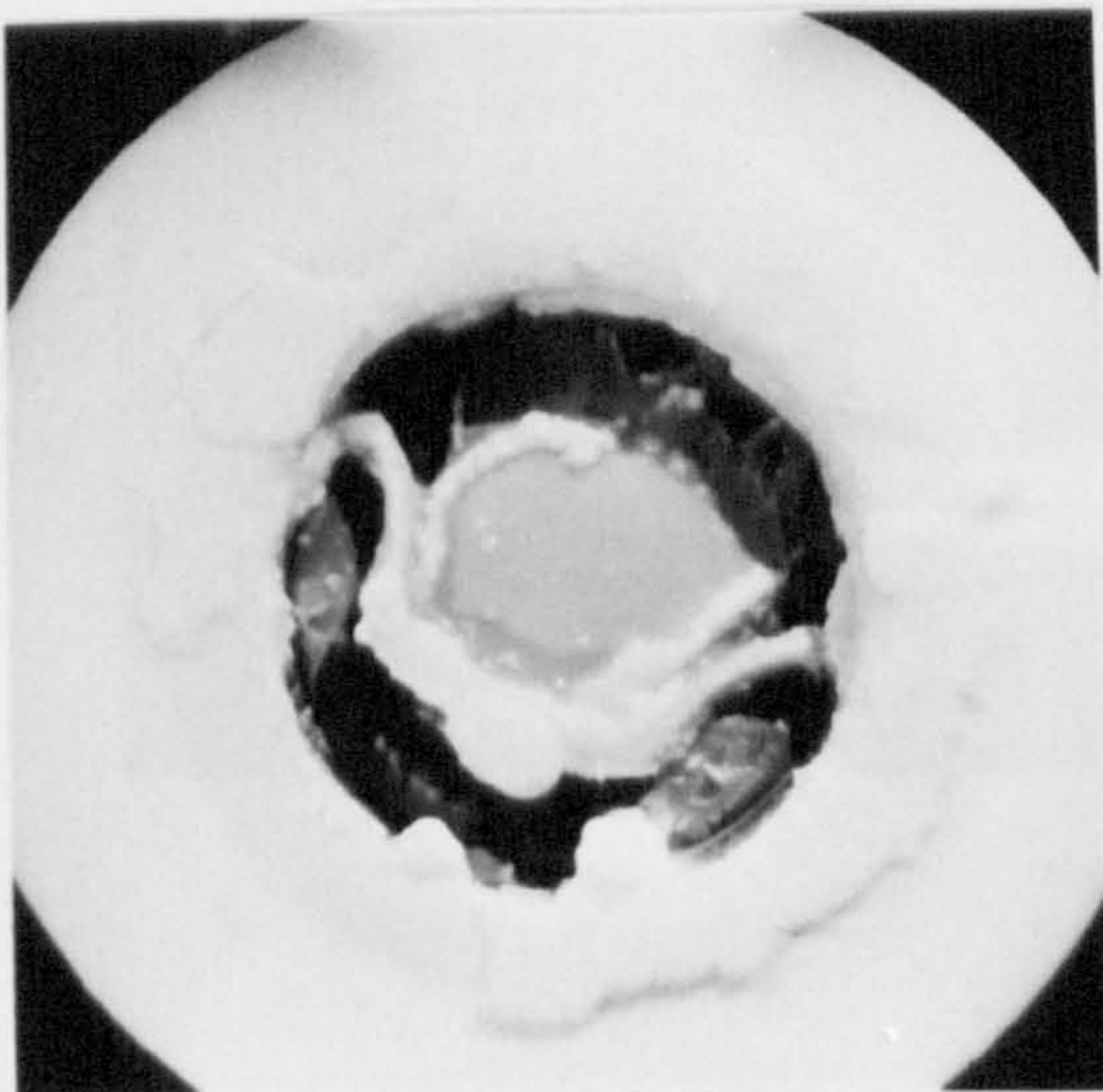


Upstream View (Run 2)

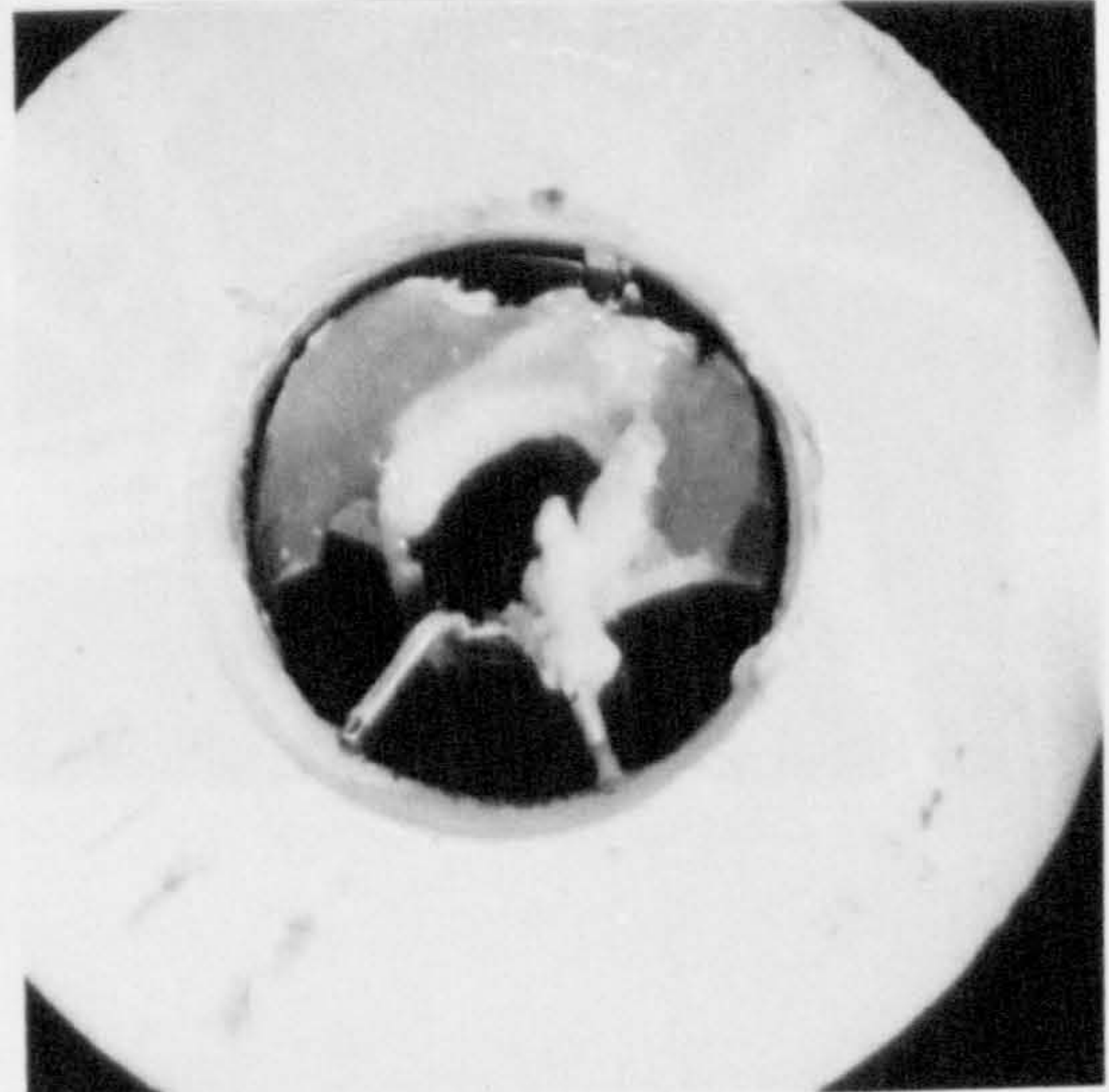


Downstream View (Run 2)

Fig. 29 Photographs of Clot around Bjork-Shiley Valve in 3l/min Steady Flow



Upstream View



Downstream View

Fig. 30 Photographs of Clot around Bjork-Shiley Valve in Heart Chamber (aortic position, 2l/min pulsatile flow)



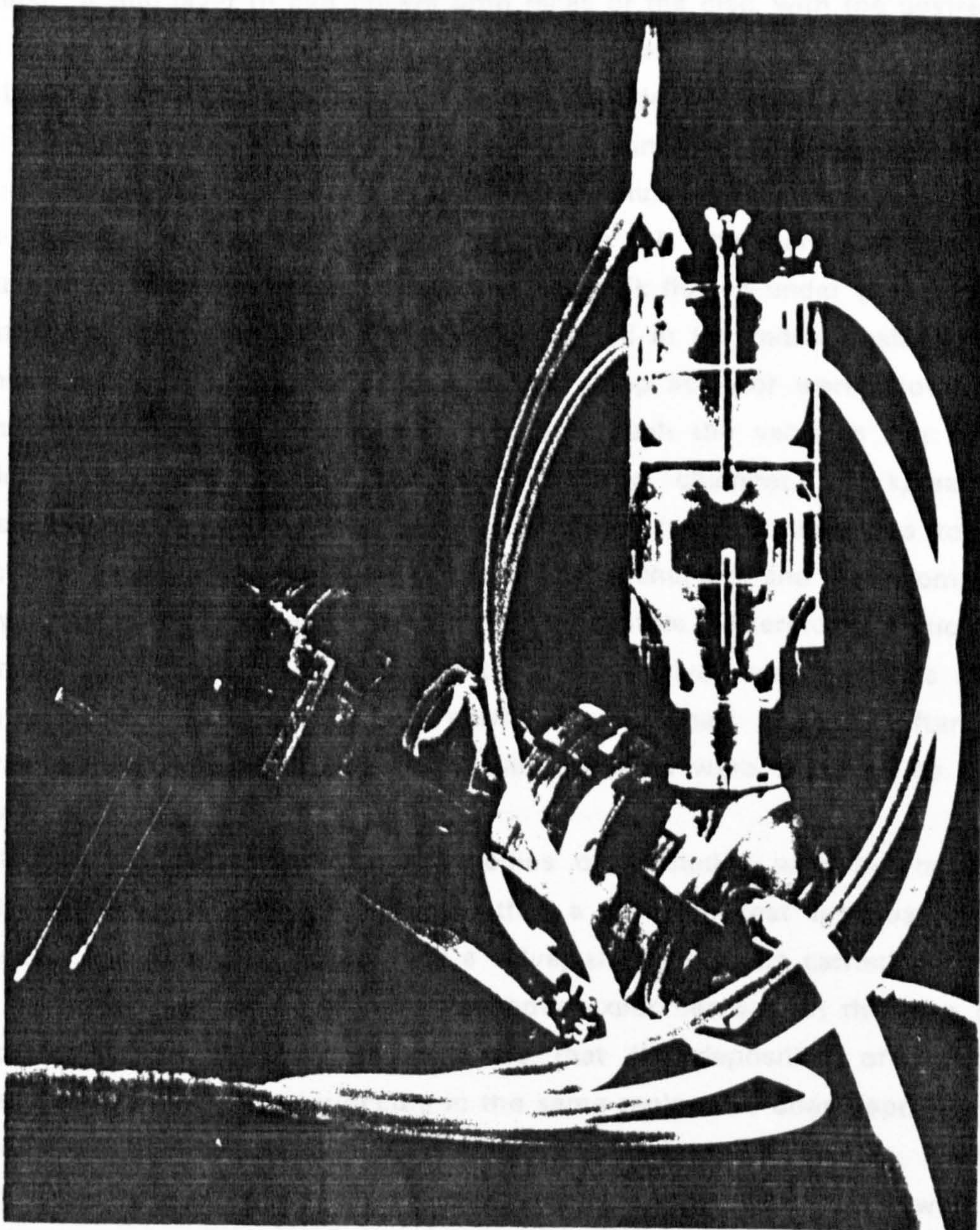


Fig. 31 Heart Chamber used by Lewis



to an increased Lee-White clotting time, thus preventing the formation of particles upstream of the valve.

The nature of the thin layer of clot around the valve in this test was similar in many respects to that observed by Lewis in his artificial heart chamber (Fig. 31 ). Figure 30 shows the coagulum deposited around a Bjork-Shiley valve in the aortic position where the mean residence time of the activated milk is of the order of 12-15 seconds (The lag phase in this repetition of Lewis's experiment is the same as that in the case of the bodies of revolution, i.e. 30 seconds). A thin layer of clot covers both faces of the disc, with the upstream strut, in particular, being the site of a larger thrombus.

In Lewis's experiments the milk flow was divided in two upstream of the heart chamber, rennet being injected into one stream and  $\text{CaCl}_2$  into the other. Each of these streams was again split in two, thus providing four milk inlet flows to the atrial chamber. Swirling of milk in this chamber would provide adequate mixing of the injected reagents. The milk flowed under gravity from the atrial chamber, through the test valve located in the mitral position, into the pumping chamber as the bellows in the pump actuator were allowed to expand. During systole this milk is forced through the valve in the aortic position and thence to the drain. In this heart chamber, the spread of residence times is greater than in the cylindrical test chamber, due to the vortical nature of the flow as milk enters the atrial chamber and the incomplete emptying of the ventricular chamber during systole. Therefore, particulate material may be present upstream of the 'aortic' valve, even with this short mean residence time. Indeed, on examining the heart chamber after the experiment, both the atrial and ventricular chambers were found to be lined with a thick layer of milk curd.

Following the experiments on the bodies of revolution with the medium length test chamber, it would appear that a standard test for coagulation (caused by the nature of flow around a valve) should best be carried out with such a shorter test section. From the above experiments, with the long test section, it is only possible to conclude that the deposition of particles suspended in a pulsatile flow occurs in the same regions as does deposition in a steady flow at flowrates typical of those prevailing during systole. Further experiments are necessary to ascertain whether the type of clot found with the mean residence time significantly less than the reaction induction period is similar in the case of both pulsatile and steady flow around the Bjork-Shiley valve.



### 6.3 Experimental Difficulties

In all of the experiments reported in the previous two sections a fairly uniform layer of clot was found to deposit on the walls of the test chamber. During disassembly of the apparatus this clot would often become detached over quite large areas so that care had to be taken in separating the pipe sections. A technique of slowly draining the residual milk through the flange at the base of each section in turn, was developed. On opening the first flange, and removing the uppermost test section, the wall clot in the lower section was often observed to become detached from the wall. Two spatulas were then used to hold this wall clot in position whilst the test object was removed, in an attempt to prevent collapse of the wall clot onto the object. In all cases it was thus difficult to determine whether there was a variation in the thickness of wall clot in the region of the test piece, the only exception occurring in the case of the disc and upstream-apex cone with the medium-length test chamber, where a thinning of the wall clot was observed about 1cm downstream of the object, i.e., 3cm from the flange. This thinner clot appeared to remain more intact and was less susceptible to dislodgement from the wall, possibly because there was less weight of clot to cause it to fall. Observed variations in the thickness of clot in the region of the flange, itself, however, were thought to be unreliable as the clot was torn at this point on splitting the test chamber, and slippage of clot from the upper section occurred in a number of cases.

Due to the invariable presence of this clot on the test chamber walls, and the difficulties encountered in determining its thickness, two proposed kinds of tests were abandoned. The Gott vena caval ring test, with the inclusion of an orifice, was to have been simulated by the insertion of an orifice into the test chamber. Changes in thrombogenicity would have required assessment of the change in thickness of the wall clot. This was not thought possible, especially as the area of interest would have been close to the flange. Similarly, it was deemed impractical to repeat Vorhauer's second set of experiments, assessing the effects of turbulence downstream of the test objects, since this, too, would have involved measuring the thickness of the wall clot, and possibly its weight, a distance of 3cm downstream of the test object. For the same reasons, it was also not possible to investigate coagulation caused by a sudden expansion or contraction in the flow.

An experiment was carried out to determine how soon after mixing this wall clot starts to appear. Evidence of a thin layer being deposited in the conical diffuser suggested that the best approach would be to run the milk mixture along the silicone rubber tubing directly from the mixing point to the drain, without involving the test chamber. The distance along the tube that wall clot

first appears could then be determined. By using this narrow tube, it was possible to maintain a plug-flow regime, without recirculation (as might occur in the diffuser), and to amplify the distance over which the first signs of clotting should be observed (by increasing the axial velocity).

In this tube (12.7mm i.d.), at a flowrate of 2l/min, wall clot was first observed 30cm downstream of the mixing point. If the explanation for this early appearance of clot is acceleration of the clotting process (caused perhaps by increased diffusion due to agitation), the delay time of the reaction must have been reduced to 1 second. This would then imply that in the test chamber (medium-length), where the time delay prior to reaching the object is about 10 seconds, the reaction should be fairly advanced in the region of the object. Observation of the fluid arriving at the drain reveals that the bulk fluid is still of a normal fluid consistency, so no such acceleration of the clotting process can have occurred in the stream as a whole. Two other explanations are possible. The clot could be forming from a reaction between the rubber tubing and the milk; or the clot is forming within the slower-moving boundary layer at the wall. The former is unlikely, in that milk without rennet added does not clot at these temperatures on the wall. The latter is the more plausible explanation.

If, then, this early deposition originates from slow moving strata of the wall layer, there arises the question why no deposit is formed upstream by that fluid closer to the wall which is moving even more slowly. This lack of deposit in the first 30cm following the point of mixing is not due simply to a micelle exclusion layer at the tube wall. If the flow is assumed to be fully turbulent, then the layer of fluid closest to the wall that might be expected to have a residence time of at least 20 seconds, at a distance of 30cm downstream of the point of mixing, is  $3\mu\text{m}$  from the surface. The micelle exclusion layer on the other hand, will only be approximately  $0.3\mu\text{m}$  thick (micelle diameter =  $50 - 300\text{nm}$ ). Two other explanations are possible for this apparent lack of coagulum in the first 30cm following mixing: The milk and rennet may not be well-mixed at the outlet of the 'Y' junction, so that diffusion of rennet into the boundary layer may limit the reaction in this region; or the quantity of coagulum deposited may vary in relation to the thickness of the layer at the wall in which particulate matter has started to appear, thus giving an apparent absence of deposit in this region following an experiment of limited duration.

The experiments reported in this chapter would strongly suggest that stasis might indeed be the overriding criterion in determining the sites of thrombus deposition, though the possibility of turbulence near the wall, or impingement of fluid onto the wall, contributing to this deposition has not been ruled out. A simple experiment was sought to determine the effect, if any, of turbulence on both the rate and nature of the clotting reaction.



## Chapter 7. A Modified Lee White Test

### 7.1 The Lee White Test and its Adaptation for Milk

In the standard Lee White test<sup>94</sup>, for determination of the clotting time of blood, 1cm<sup>3</sup> of venous blood is drawn by syringe and injected into a test tube (Widal tube) of 0.8cm diameter (both of which having been rinsed previously with saline). The time of insertion of the blood into the test tube is noted. A plug is placed in the test-tube and the tube is inverted every 30 seconds until the blood will no longer flow in the tube. This time is noted and the clotting time thus calculated. The test is normally conducted at room temperature.

Lewis carried out a similar test with milk. He injected 1cm<sup>3</sup> of milk into a 0.8cm diameter test-tube, added 10μl of each of essence of rennet and saturated aqueous CaCl<sub>2</sub>, plugged the test-tube and inverted the tube every 30 seconds until the milk would no longer flow. The clotting time was thus estimated.

The clotting time of milk with these rennet and CaCl<sub>2</sub> concentrations is normally between 30 and 90 seconds for temperatures ranging from 40°C to 30°C. Thus this technique, as it stands, is not accurate enough to detect small changes in clotting time. A variation of this technique, inverting the test-tube every 15 seconds, was thus tried. The clotting time for a range of rennet and CaCl<sub>2</sub> concentrations in milk was evaluated using both rates of inversion. No significant differences in clotting time were observed between the two inversion rates. Having established that doubling the inversion rate had no influence on the Lee White clotting time, the test was repeated using a five-fold increase in milk volume, in a 1.2cm diameter test-tube (This allowed for more accurate additions of rennet and CaCl<sub>2</sub>). Again, this volume change had no effect on clotting time. This test, with 5cm<sup>3</sup> of milk and inverting every 15 seconds, was used as a preliminary test before each of the runs of the large-scale apparatus, to test for enzyme potency. A water bath was used to ensure a constant temperature in each test, the milk being left to reach the water bath temperature for 15 minutes before additions of first CaCl<sub>2</sub> and then rennet were made.

### 7.2 Modification to Test for the Effects of Agitation

The influence of shear on clotting is unknown, and one possible reason for milk clotting in certain locations in the flow before the bulk mixture coagulates is that the time history of the fluid, entering such a region, includes a period of high shear of above average duration, leading to local acceleration of the clotting process. This is particularly likely to be the case in blood, where

damage of blood elements due to shear is known to be linked to both the magnitude of shear and the exposure time to this shear. For instance, this might explain why coagulum forms preferentially downstream of the net in Hladovec's experiment, as the fluid in this region has just been exposed to high shear on passing through the net, and high intensities of turbulence also exist within this wake region itself.

Since shear might affect the rate of coagulation, if coagulation is the result of diffusion-controlled binary collisions (Smoluchowski<sup>197</sup>), it is interesting to ask at what stage, if any, will shear influence the rate of coagulation. A simple experiment was therefore devised, based on the Lee White test, to attempt to answer this question.

The Lee White test was performed as normal, using a weaker clotting mixture (0.4%vol injections) and a 30 second inversion rate, except that during one of the 30 second periods, when the milk would normally be stationary, the test-tube was shaken vigorously. After shaking, the test was continued as normal. By varying the starting time of the particular 30 second period chosen, from that immediately following mixing of the reagents through to the period when the mixture had normally solidified, it was hoped to ascertain the effect of this gross agitation at various points during the clotting reaction. In these experiments, the time at which particles were first seen to form in the milk ( $>100\mu\text{m}$  diameter), the time taken for the mixture to cease to flow and the macroscopic structure of the formed clot were all noted. The results are given in Table 5.

In the normal test the first sign of small particles in the fluid occurred after 1 minute, with larger particles (1mm diameter) not being observed until a further minute had elapsed. The test was complete after 2.5 minutes, when the milk in the tube had clotted solid. After the clot had contracted (20 minutes later) a very smooth plug of clot was left in the tube, now detached from the wall and surrounded by clear liquid.

Shaking of the test-tube during the first minute after addition of the reagents had no influence on the observed pattern of clotting, the same observations being made as for the standard test.

When the tube was shaken during the period following the first observable signs of particles in the milk, a thick wall clot was observed after a further delay of 30 seconds (i.e. 2 minutes from commencement of the test). This clot built up to form a solid plug after 3 minutes. When contraction occurred, a thick clot was left adhering to the wall, and a smaller clot, spongy in nature, was found in the centre of the tube. The overall clotting time in this test was not changed but the nature of the deposit was radically different. This experiment was repeated several times to ascertain that this phenomenon is



**Table 5**

**Effect of Agitation on the Clotting Behaviour of Milk**

Time at Start of 30sec Agitation Period / s	Time First Particles Observed /s	Time (s) for Complete Coagulation	Observations of Formed Clot
no agitation	60	150	Smooth plug of clot
0	60	150	Smooth plug of clot
30	60	150	Smooth plug of clot
60	60	150	Clot forms preferentially on wall of tube
90	60	150	Loose spongy plug of clot
120	60	180	Loose spongy plug of clot
150	60	150	Clot broken up on shaking
No agitation, milk carefully inverted	60	150	Smooth plug
Agitated before enzyme addition	60	150	Smooth plug
No agitation, N <sub>2</sub> displaces air	60	150	Smooth plug
No agitation, but preformed clot added	60	150	Fairly smooth plug



indeed reproducible, as opposed to being an single spurious result. It would appear therefore that gross agitation at this stage leads to the clotting reaction taking place preferentially at the solid-liquid boundary as opposed to the bulk fluid.

The experiments in which agitation occurred after 1.5 minutes, i.e. after the coagulation reaction was well under way, all resulted in a fairly solid lump of clot after 2.5-3 minutes. This lump of clot never adhered to the wall and was observed to drop *en masse* as the tube was inverted. After contraction of the clot, a white spongy coagulum was left surrounded by a faintly yellow liquid.

In these experiments the effects of an increased air-liquid interfacial area and agitation cannot be separated. However, the fact that shaking during the first minute had no effect on the reaction time, whilst presumably increasing the proportion of air trapped in the liquid would suggest that the observed effects are due to agitation. Two further Lee White tests were performed to attempt to test for the influence of dissolved gases in the milk. In one test the milk was agitated before the addition of enzyme, the rest of the test being conducted almost as normal, with the milk being agitated slightly on inverting the tube. In the other the milk had been allowed to sit for 1.5 hours before carrying out the test and inversions of the tube were carried out slowly to minimise disturbance of the fluid. No difference in clotting time was observed.

In another test, using milk through which  $N_2$  had been bubbled to displace the air, with the milk again being allowed to settle for 1.5 hours, in contact only with  $N_2$ , a similarly careful Lee White test was performed. Again there was no observable difference in the Lee White clotting time.

Whilst these tests do not rule out the possibility that an enhanced gas-liquid interfacial area, at an appropriate stage of the reaction, may be important, it seems more plausible to suppose that agitation at this time brings particles into intimate contact with the solid surface, unto which they bond.

It was thought that this might be analogous in some ways to crystal formation from a supersaturated aqueous solution. It is known that if a supersaturated solution is placed in a glass beaker, crystal formation can be induced at the wall by scratching the wall of the beaker with a glass rod. Crystal growth will then occur preferentially in this region, ultimately leading to a projection of crystal from the wall. Whilst the formation of clot preferentially at the wall on shaking after 1 minute may be of a similar nature, growth thereafter is probably not. A Lee White test, in which some clot, already formed in a previous experiment, was added with the clotting reagents into fresh milk, showed no observable difference in clotting pattern from the normal test. Clot was not observed to form on that already *in situ* except in the final stages when the whole sample congealed.



The significance of this ability of the milk clot to adhere preferentially at a solid surface, on being agitated at the appropriate time, in relation to the experiments involving the axisymmetric test bodies is uncertain. It would appear, however, that agitation of the fluid, in the vicinity of a solid surface, at a stage in the coagulation reaction when particles, of a size visible to the naked eye, are beginning to appear, may lead to the deposition of a clot whose adhesivity to the surface is greater than if no agitation exists. Thus both stasis, of a period sufficient for the reaction to reach this critical stage, and agitation are implicated as being necessary for a strongly adhesive clot forming on the surface of any object in the flow. It is possible that other coagulation products will deposit on the surface of objects in the flow, but this experiment would suggest that they would be less adherent and therefore more easily dislodged by shearing forces.

Since stasis is implicated as a condition for adherent deposition, it is prudent to ask whether the strange deposition patterns, in the case of the test bodies in the medium length test chamber, can be explained in terms of the locations and sizes of regions of stasis around these objects in both steady and pulsatile flow. Flow visualisation studies were therefore carried out to locate such regions.



## Chapter 8. Residence Time Studies

### 8.1 Dye Injection Experiments

The simplest means of obtaining information on the gross flow pattern is that of injecting an instantaneous pulse of dye into the fluid stream and observing its progress downstream. A saturated aqueous solution of Potassium Permanganate was chosen for these experiments, because of its strong colour, in order that it might remain visible even after dilution in 1000 times its own volume of water. This was necessary, since, as already mentioned in the discussion of mixing of the reagents with milk (section 5.7), pulsatile flow promotes a limited degree of axial mixing. This axial mixing leads to a 30% spread of residence times around the mean value in the milk test apparatus, when using the same pulsatile flow settings as for the milk experiments.

An alternative solution to this axial mixing, other than increasing the concentration of the dye, might have been the use of electrolytic  $H_2$  bubbles in the flow. However, it was felt that the results of a pulsed dye injection would be more easily interpreted, from a residence-time point of view, than a continuous, or semi-continuous, release of bubbles into the flow.

The experimental technique, using the dye, was as follows. A video camera was focused onto the region of flow around the object, and a 1ml syringe, containing 100 $\mu$ l of saturated aqueous Potassium Permanganate, was used to inject dye into the fluid stream at the point where rennet is normally injected. Cold water was circulated through the equipment in place of the milk. The flowrate and pulsatile frequency were set as in the milk experiments. An audible signal was recorded on the video tape at the time of injection of the dye, with patterns caused by the dye, in the neighbourhood of the object, also being recorded on the video tape.

Later, the dye patterns were analysed. The time for the first and last visible signs of dye reaching the upstream tip of the object were recorded, along with the time for the concentration of dye to reach a maximum. Thus the mean residence time upstream of the object and the spread around this mean were measured. The times at which dye disappeared from around the object and the walls of the test chamber were also recorded, to provide a measure of the maximum residence time between the injection point and each of these regions. These times are given in Table 6. Figure 32 gives a pictorial impression of the behaviour of the dye around the objects in steady flow, whilst Figure 33 gives a similar diagrammatic view of the movements of dye, during both systole and diastole, in pulsatile flow.



**Table 6**  
**Residence Time Experiment Results.**

**a) Medium - Length Test Chamber**

Object	Flow * State	Time 1st dye reaches Upstream tip of Object /s	Time (Seconds) until Disappearance of Dye from:-	
			Bulk Fluid	Wake area
Cone (Down.-Ap.)	S	6	12	20
Cone (Down.-Ap.)	P	7	12	13
Cone (Up.-Apex)	S	7	13	25
Cone (Up.-Apex)	P	7	12	13
Disc	S	6	12	20
Disc	P	6	12	13
Sphere	S	7	13	30
Sphere	P	6	12	20
Tear-drop	S	6	12	40
Tear-drop	P	7	12	26
Bjork-Shiley	S	6	15	30+
Valve	P	7	15	30+
Edinburgh	S	7	15	30+
Valve	P	7	15	30+

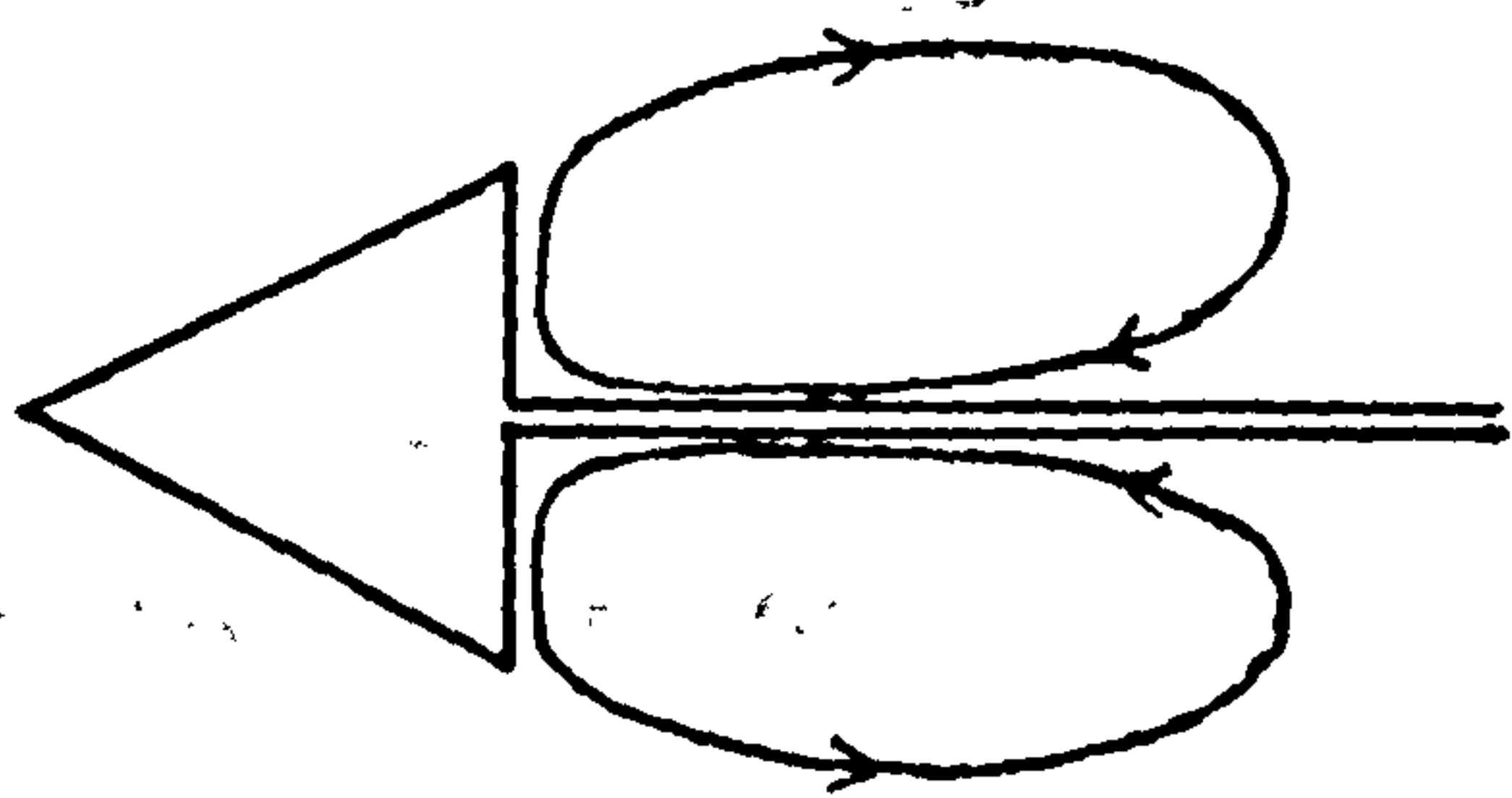
**b) Short Test Chamber**

Object	Flow * State	Time 1st dye reaches Upstream tip of Object /s	Time (Seconds) until Disappearance of Dye from:-	
			Bulk Fluid	Wake area
Tear-drop	S	1	3	15
Tear-drop	P	1	4	15

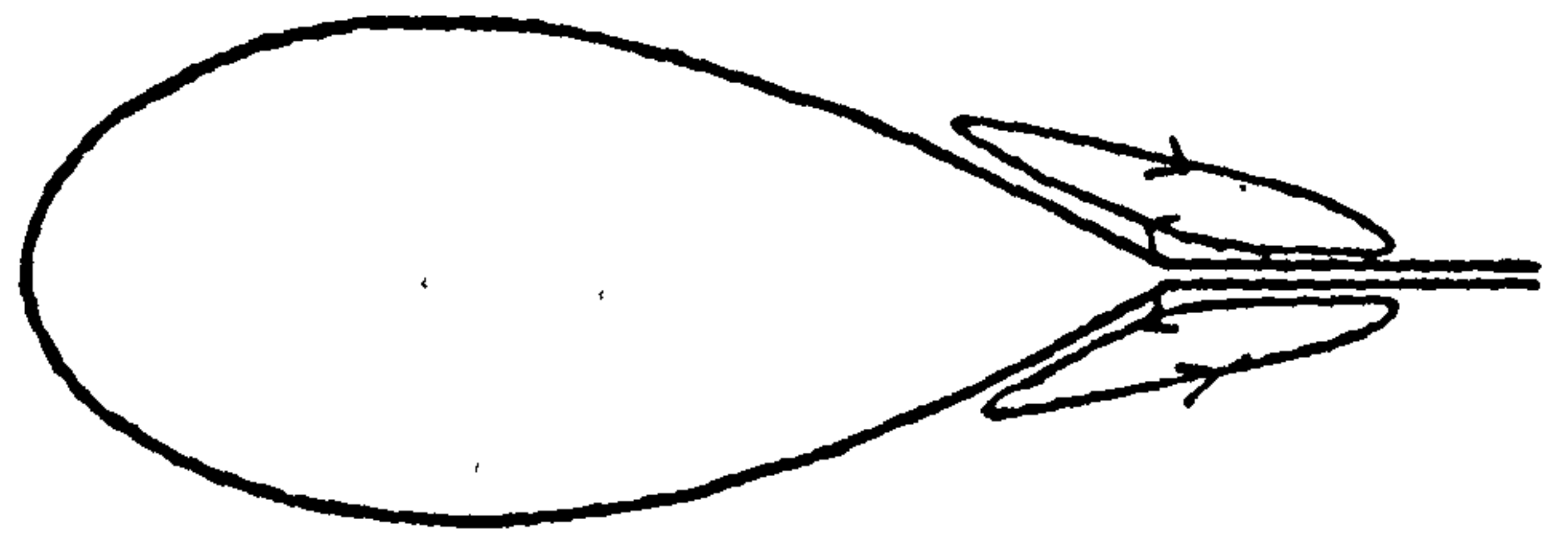
Fig. 32 Schematic Diagrams of Vapour Meters, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000

\* S = Steady ; P = Pulsatile.

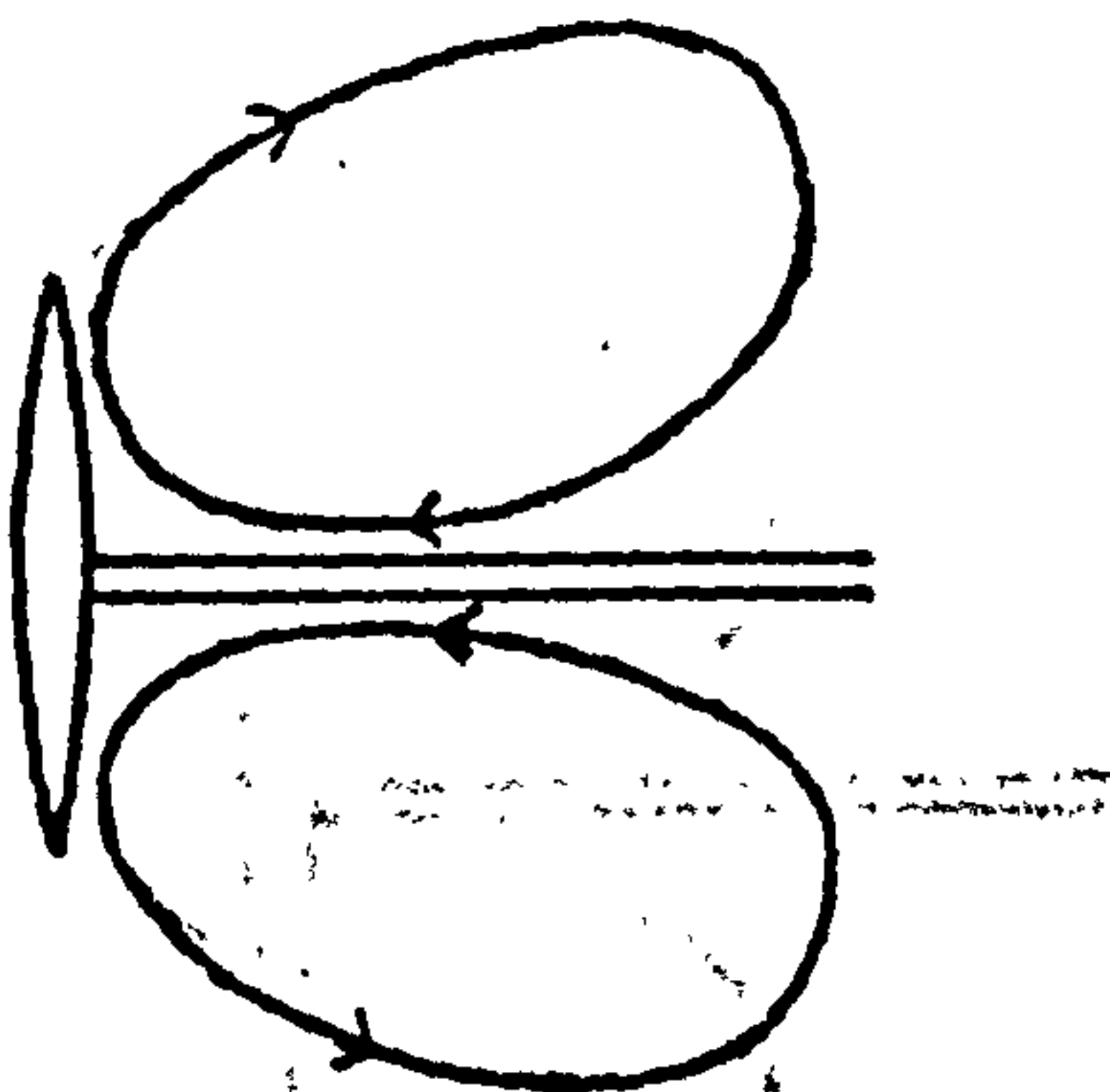




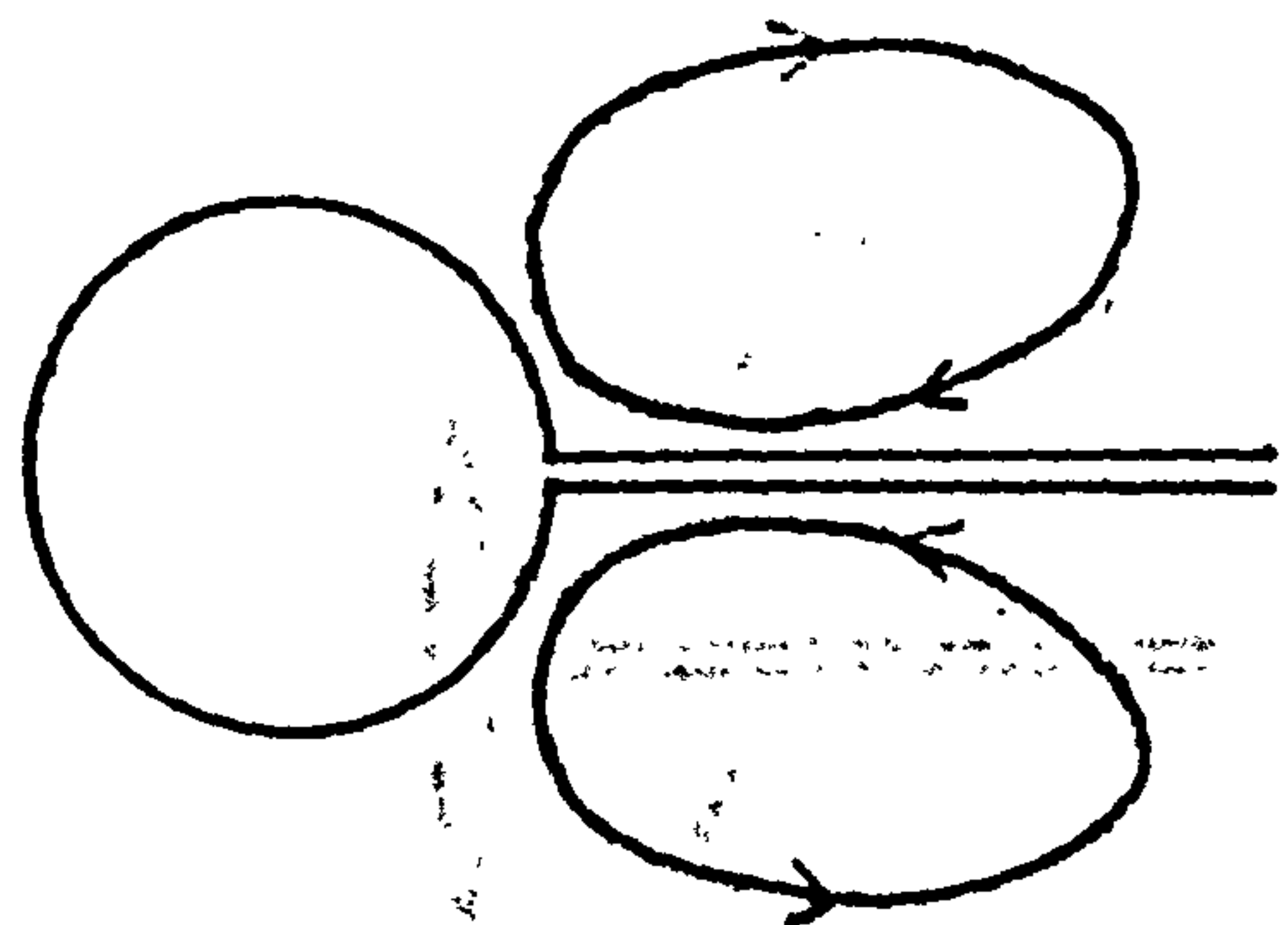
a) Upstream Apex Cone



b) Tear-drop



c) Disc



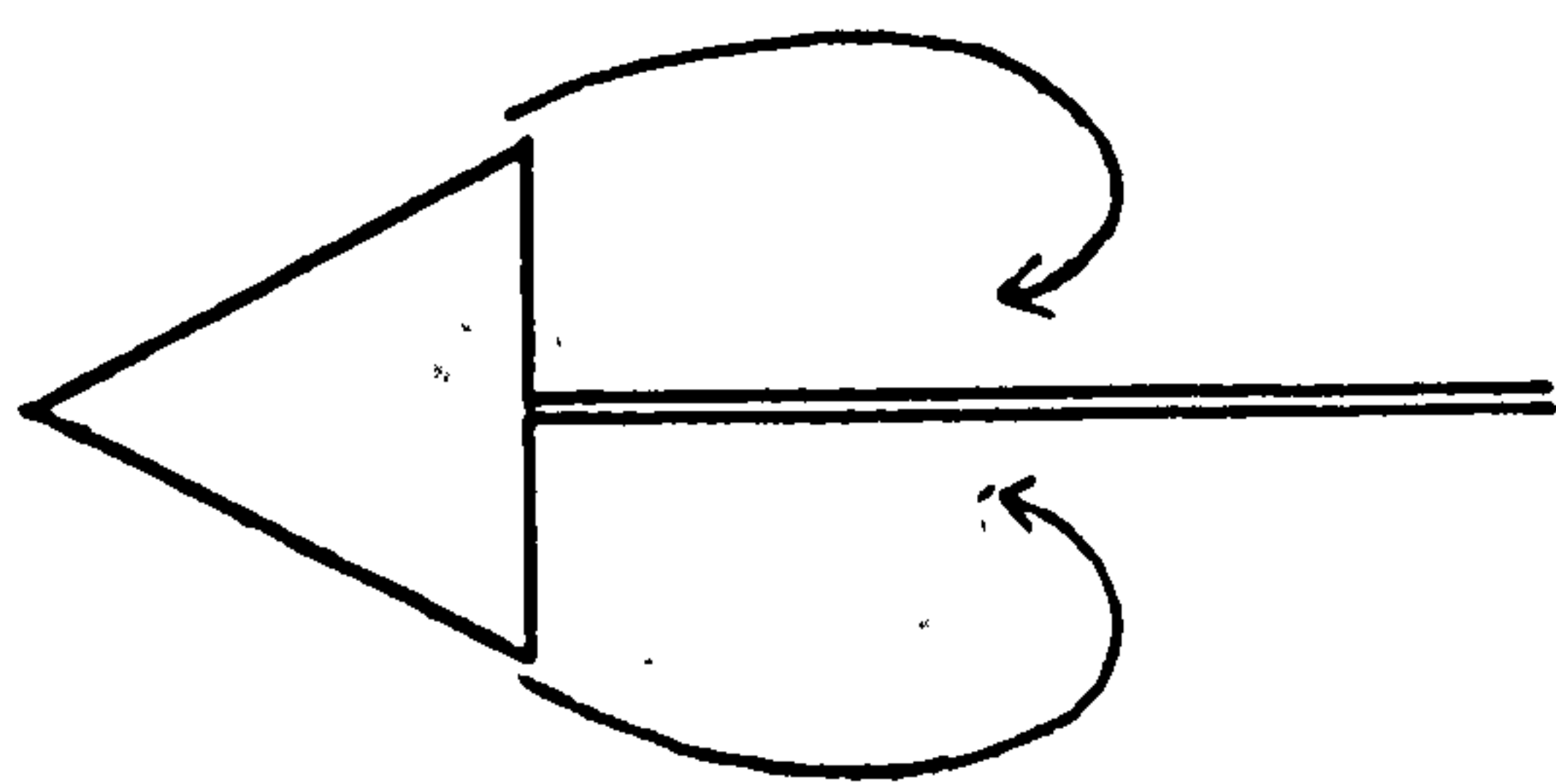
d) Sphere

**Fig. 32 Schematic Diagrams of Wake Vortices in Steady Flow**

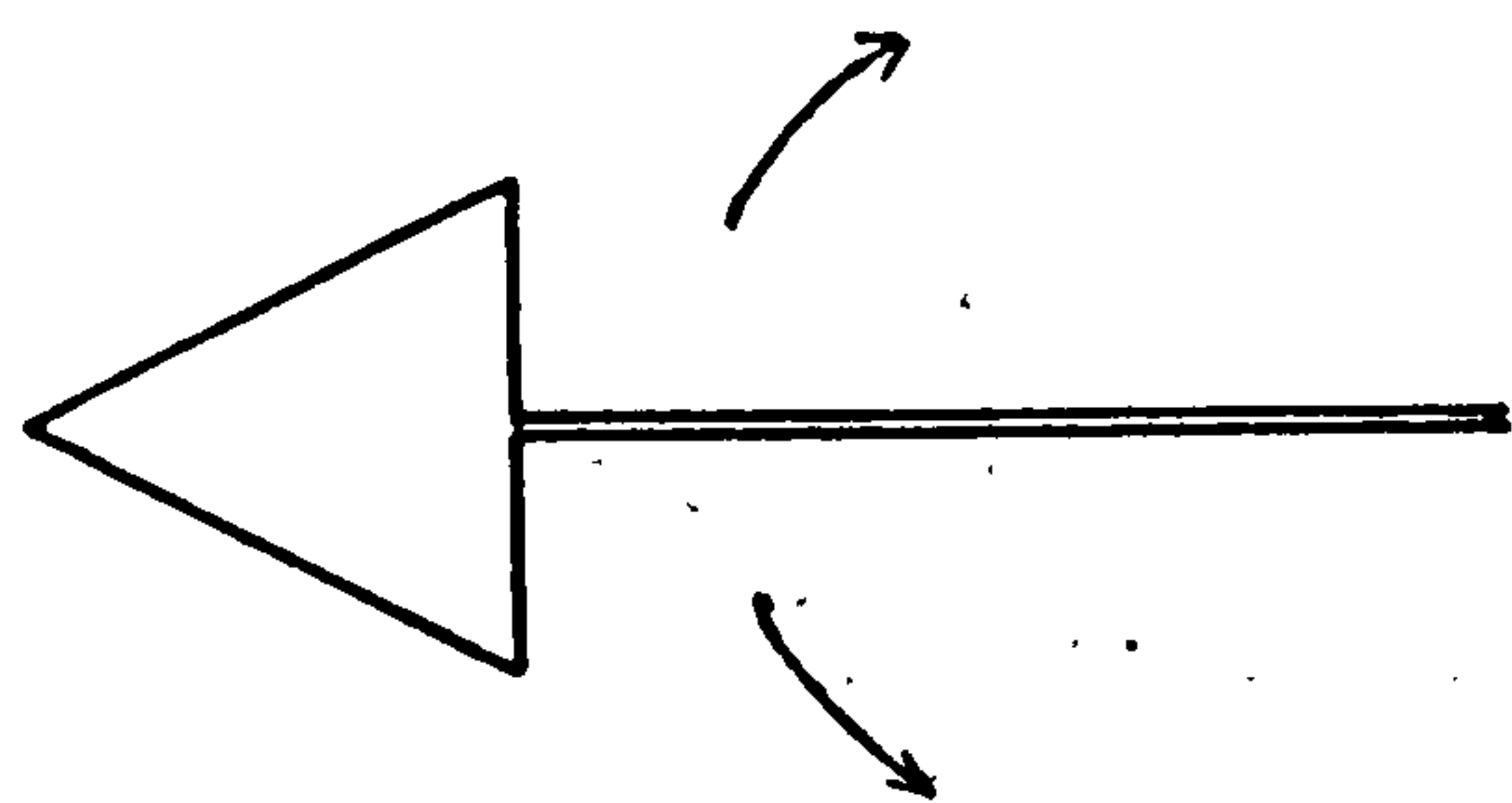
Fig. 32

Schematic Diagrams of Wake Vortices in Steady Flow



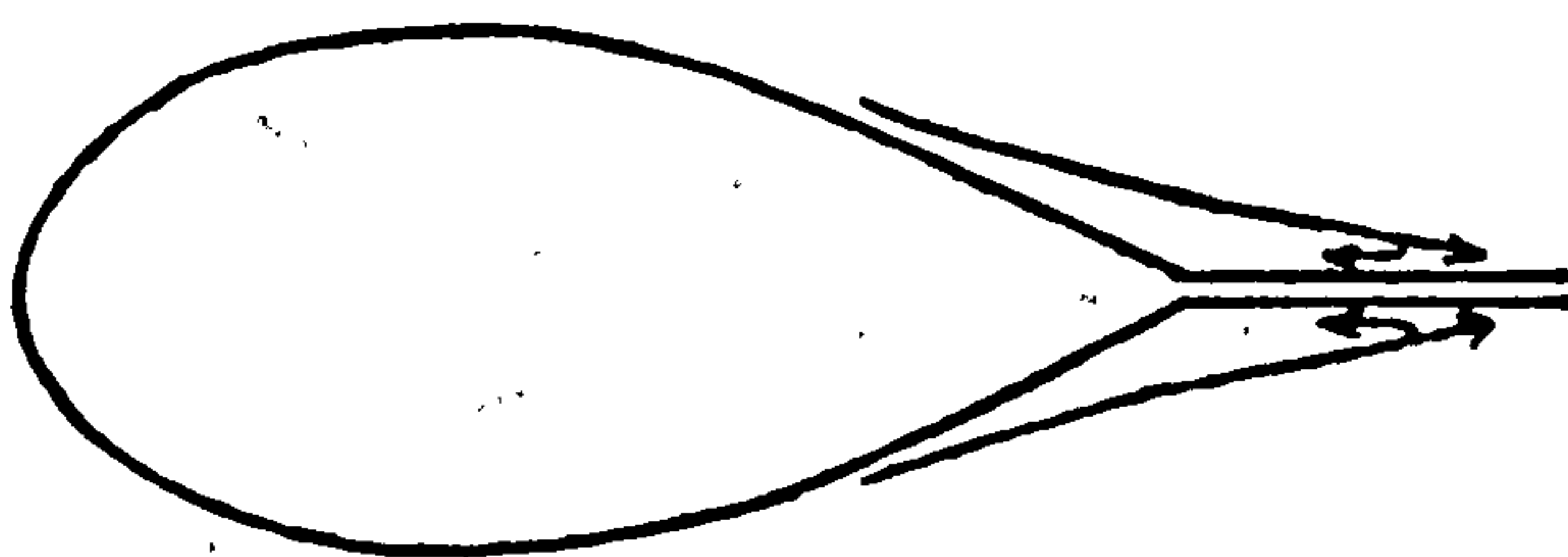


Systole

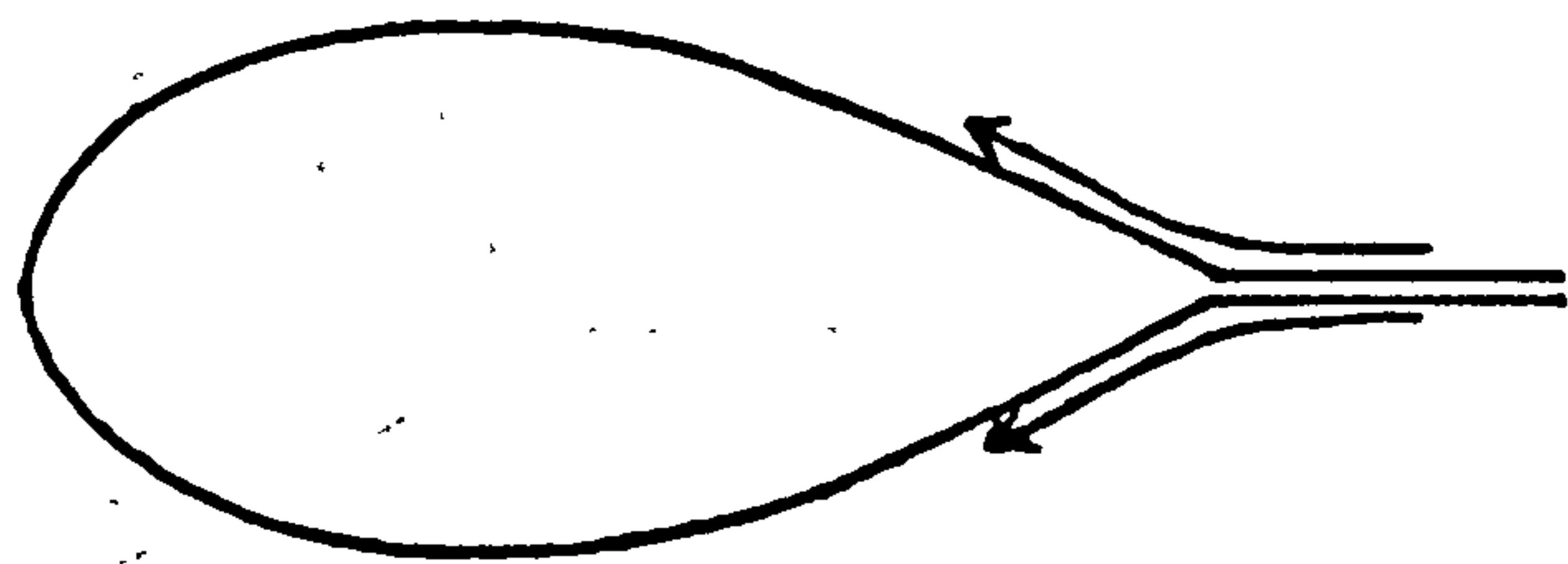


Diastole

a) Upstream Apex Cone

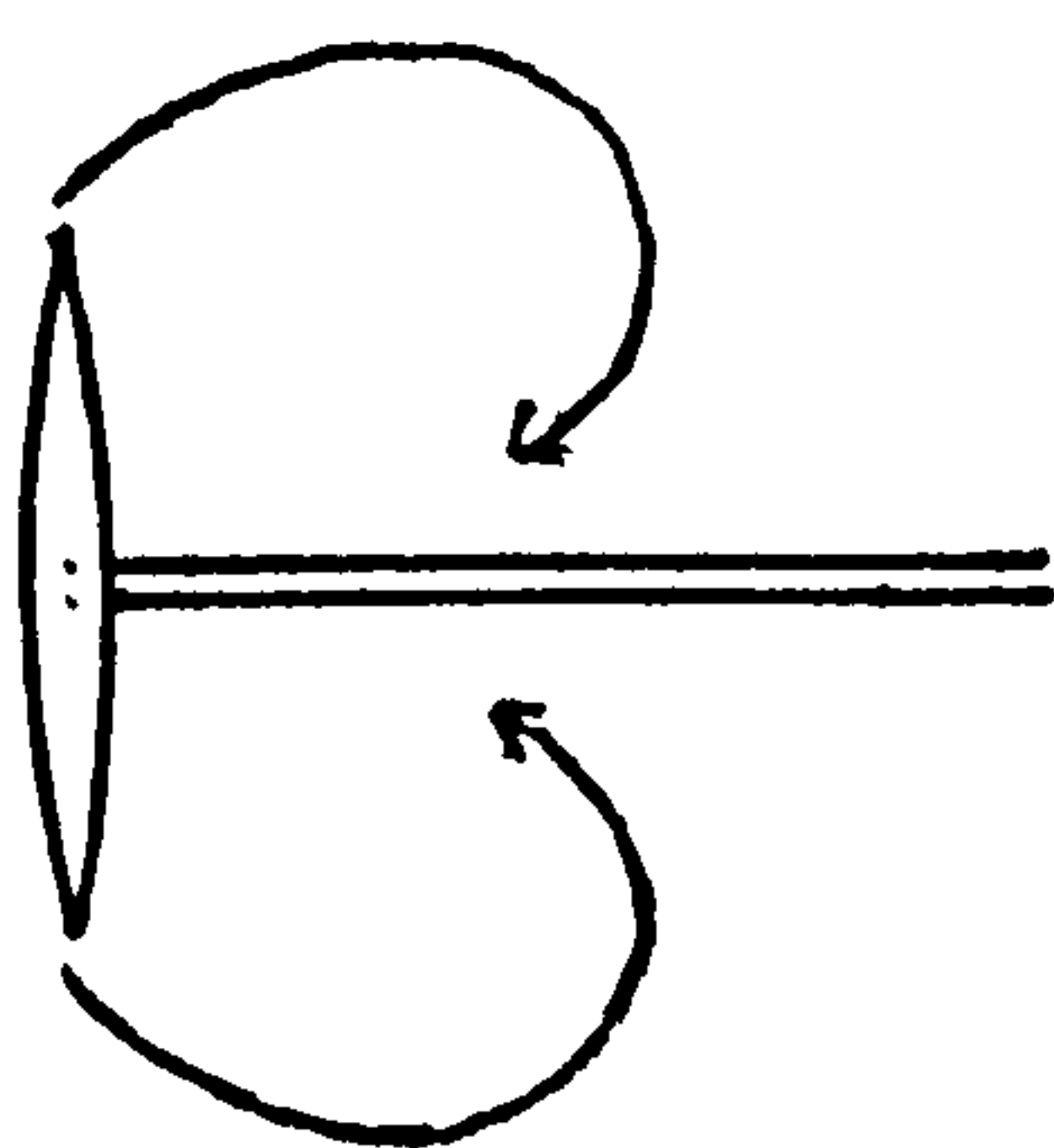


Systole

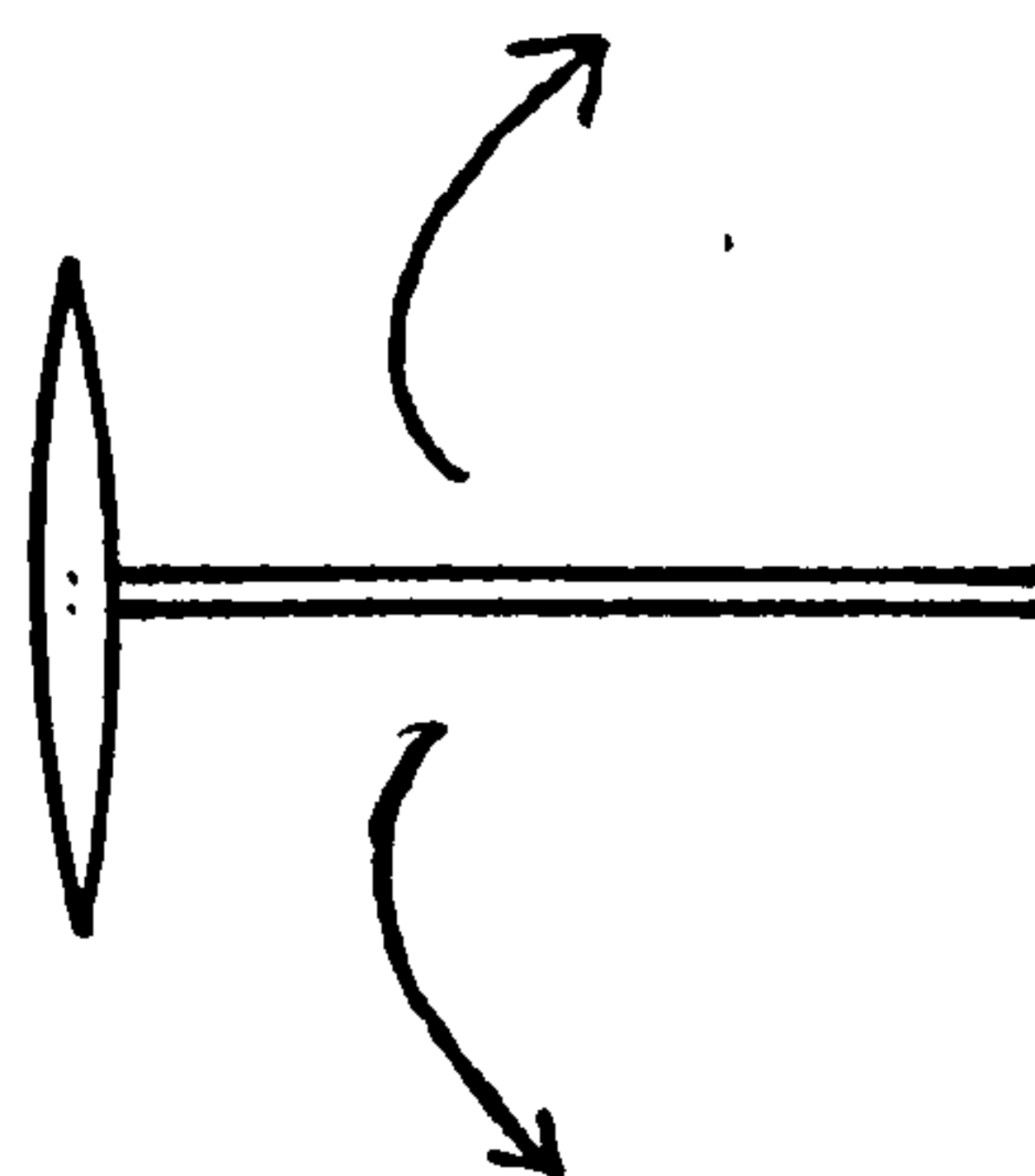


Diastole

b) Tear-drop



Systole

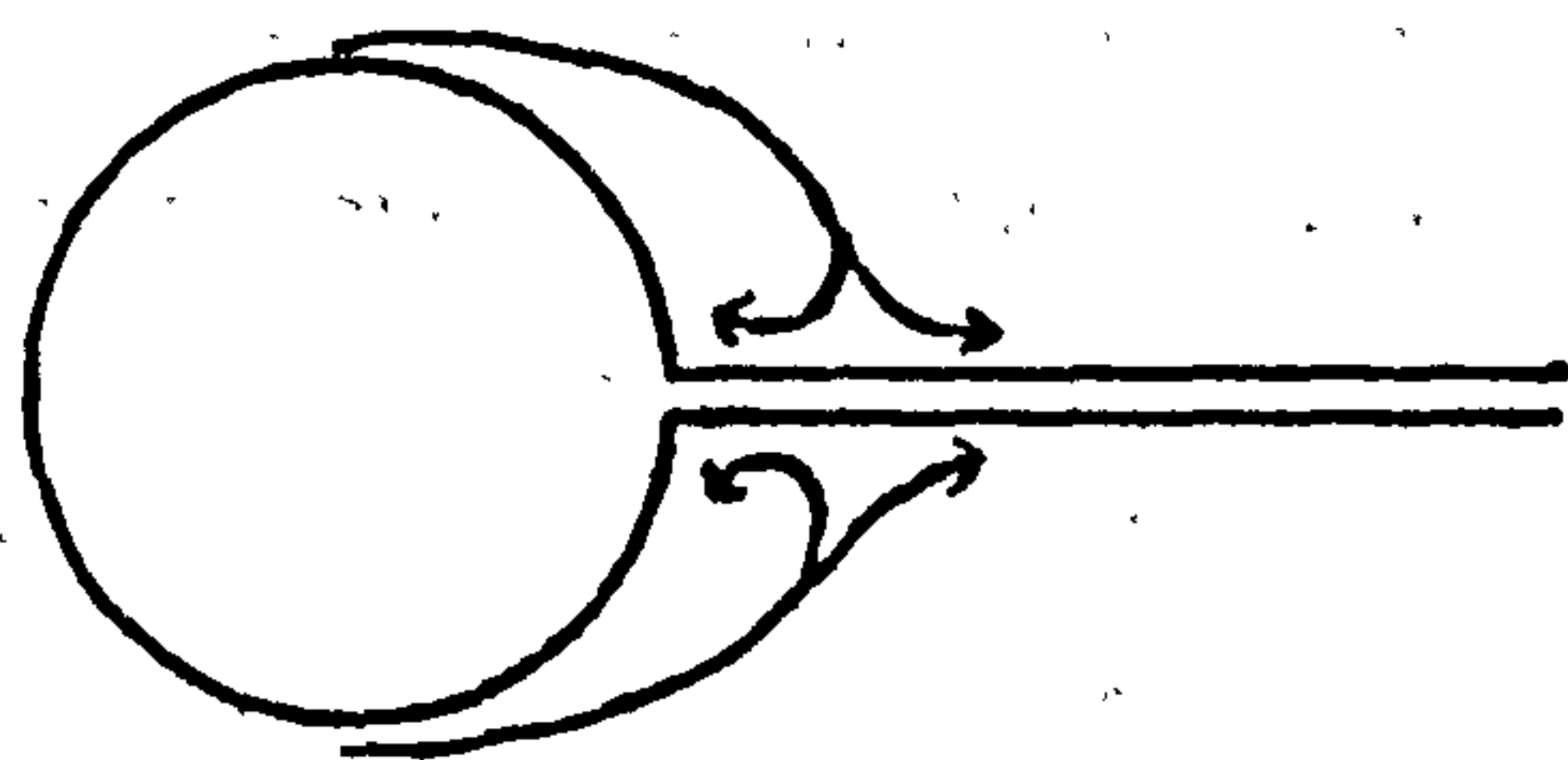


Diastole

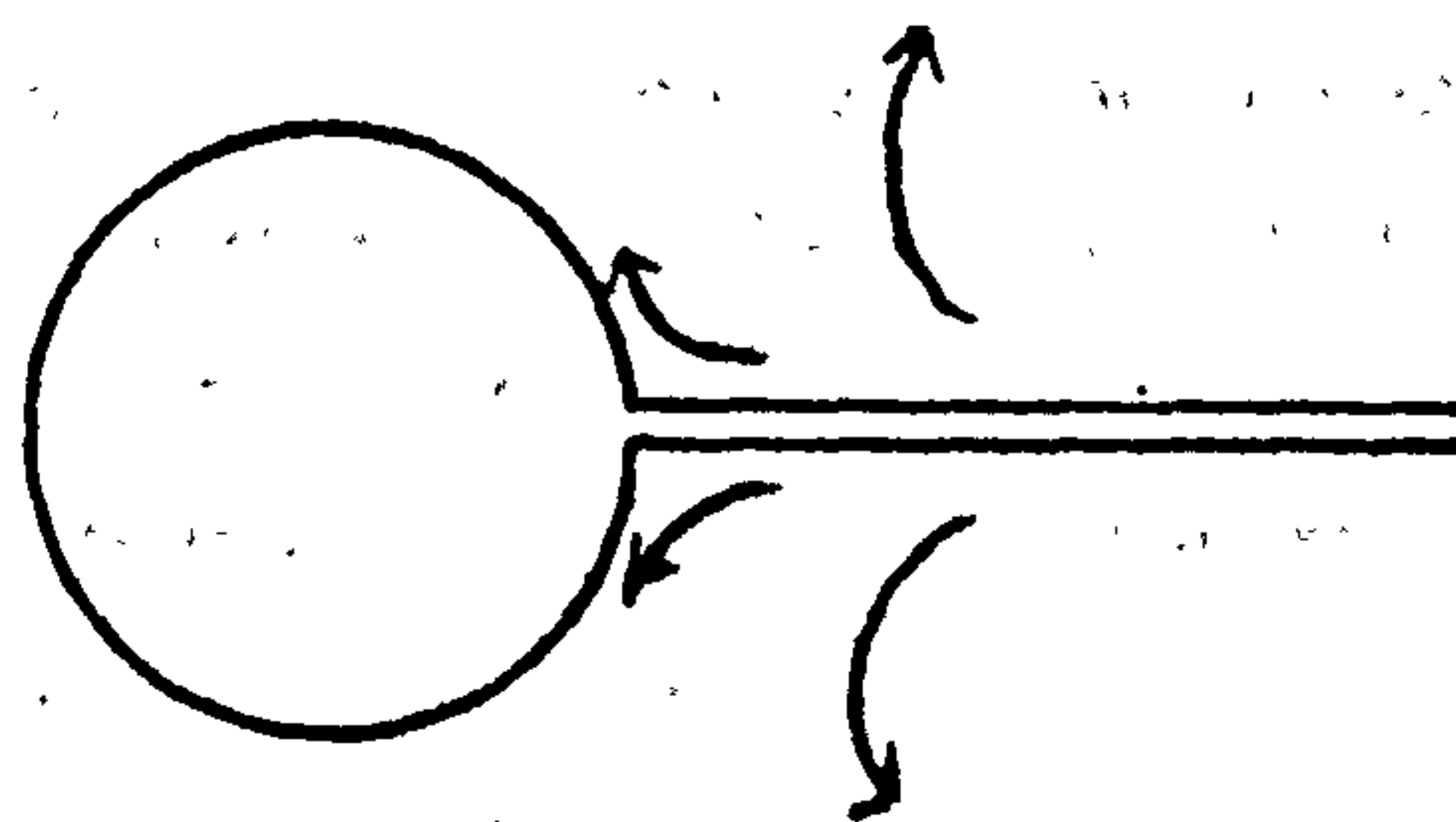
c) Disc

Fig. 33 Schematic Diagrams of Dye Movement during Pulsatile Flow



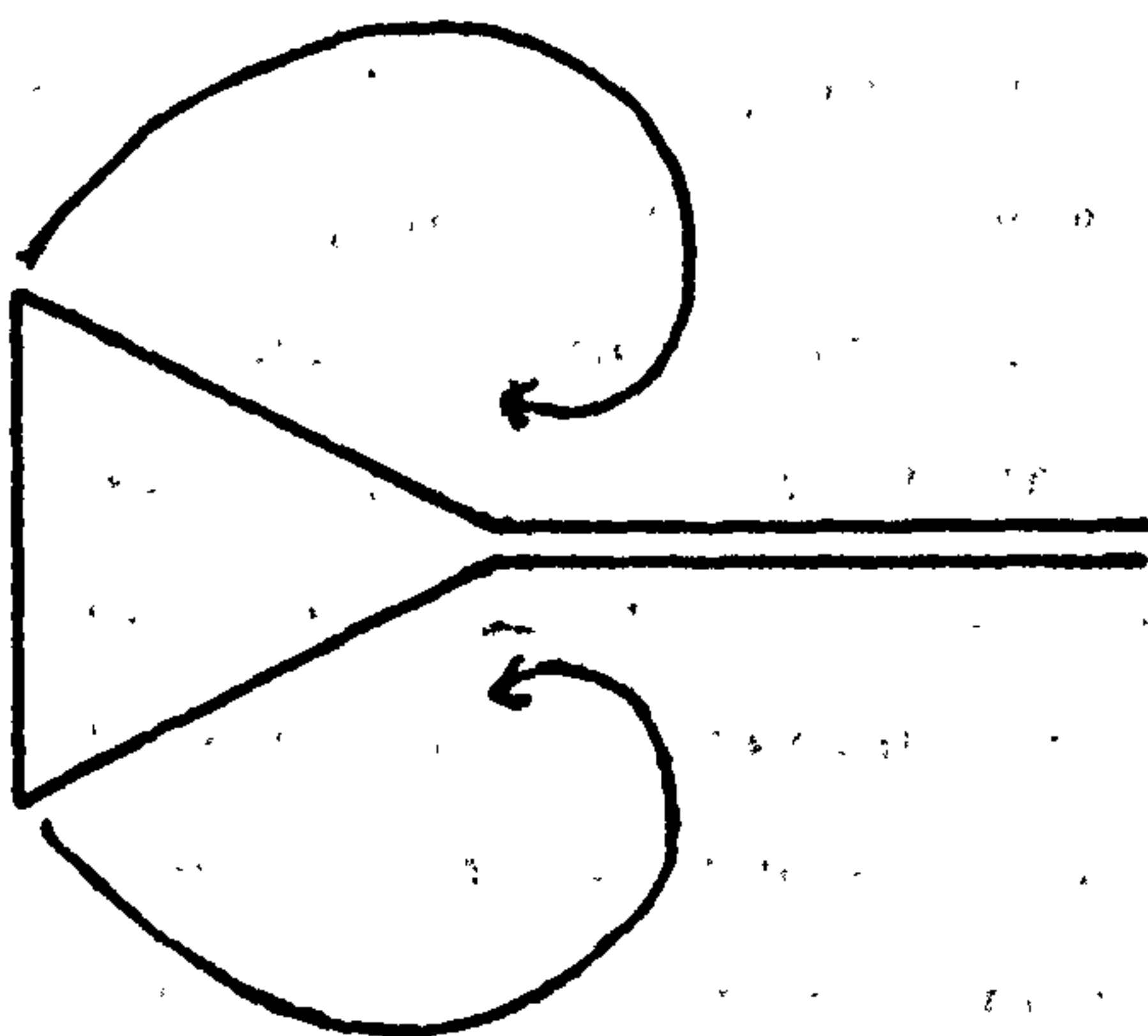


**Systole**

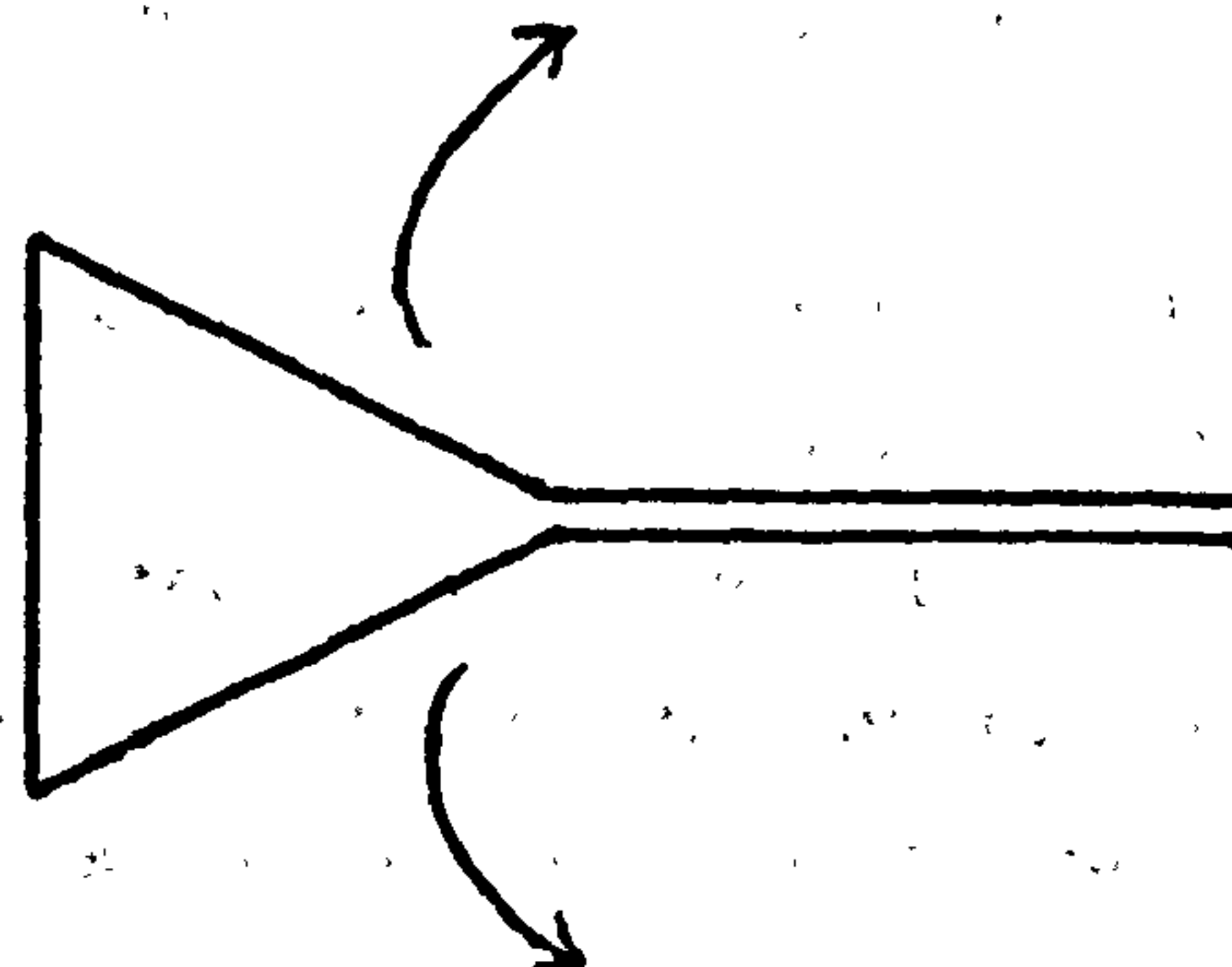


**Diastole**

**d) Sphere**



**Systole**



**Diastole**

**e) Downstream Apex Cone**

**Fig. 33 Schematic Diagrams of Dye Movement during Pulsatile Flow**



For the medium-length chamber the mean residence time of the fluid between the point of injection and the upstream face of the object was  $9 \pm 3$  seconds. For the short test section this mean residence time in the region upstream of the test object was  $2 \pm 1$  seconds. As expected, the choice of object had no influence on these values.

The time, after which dye was no longer visible in the vicinity of the object varied between 13 seconds and 40 seconds, depending on the shape of the object and whether the flow was pulsatile or steady. For all the objects tested in the medium-length test chamber under steady flow, this time for total disappearance of dye, was at least 20 seconds; the tear-drop having the longest period (40 seconds) and the disc the shortest (20 seconds). The times for the ball and the upstream-apex cone both lie between 25 and 30 seconds. The ordering of the objects as far as milk clotting propensity in their wakes is concerned, is upstream-apex cone (0.095g), tear-drop (0.080g), disc (0.071g) and sphere (0.043g). Thus, there does not appear to be a direct correlation between the residence time in the region of the object and the quantity of clot deposited in these steady flow experiments.

With pulsatile flow in the medium-length test chamber, the time after which all colour had disappeared from the vicinity of the object varied between 13 seconds, for the disc and cone (both upstream and downstream apex orientations), and 26 seconds for the tear-drop; the time for the sphere being 20 seconds. The ordering of the objects as far as the weight of clot formed on their downstream portion is concerned is tear-drop (0.090g), downstream-apex cone (0.074g), sphere (0.073g), disc (0.027g) and upstream-apex cone (0.006g). With both the disc and the downstream-apex cone much of the "downstream"-clot weighed was possibly a continuation of the upstream clot extending back over the lip of the object. In the photographs a smooth deposit is not evident for either of these shapes. It is likely that the lack of clot, at least in the case of the cone, is due to the absence of a region of stasis, the milk mixture not having attained a coagulable state until at least 15 seconds have elapsed.

For the tear-drop in the short test section, in both steady and pulsatile flow, the time before dye had disappeared from around the object was 15 seconds. In this experiment the milk temperature was  $4^{\circ}\text{C}$  higher, thus reducing the lag time of the reaction. At this temperature a coagulable state would be present after approximately 10 seconds.

In all the experiments with the medium-length test chamber dye remained visible in the region of the wall for 20-25 seconds, the corresponding time for the short test section being 8 seconds. These findings show that the residence time in the region of the wall is high enough to account for clotting there, at



least in the medium-length chamber.

In steady flow a wake region was present downstream of all the test-objects and it was in this region that coagulation was observed to occur. In pulsatile flow, for the objects whose downstream surfaces were found to remain clot-free, vortices formed during systole were shed immediately on the onset of diastole. Only in the case of the tear-drop and the sphere did the vortex remain even partially trapped for more than one cycle of the flow. Regarding the sphere and the tear-drop, an unexpected phenomenon occurred: A partially trapped pocket of fluid oscillated along the tail of the body, the upstream extremity of its motion coinciding with the location of the band of clot observed in the milk experiments. The extent of its travel downstream was difficult to determine accurately, the dye boundary being more diffuse here, but appeared to be about 1/2 of the length of the tear-drop for both objects. Colour persisted in this oscillating pocket for 26 seconds and 20 seconds, in the case of the tear-drop and sphere respectively, compared with 12 seconds in the bulk. It would seem, therefore, that stasis is a necessary condition for the adhesion of milk clot to the surface of the object, accounting for the absence of milk clot downstream of the cone and the disc and the presence of the clotted band to the rear of both the sphere and the tear-drop. That deposition is not found to occur uniformly along the full length of the trapped volume of fluid is an indication that stasis, whilst being necessary, is not, however, a sufficient condition for the formation of an adhering clot. Some other aspect of the flow (presumably present during agitation within a test-tube), perhaps local turbulence, must also be required for deposition.

A similar study, using both the Edinburgh tilting disc valve and the Bjork-Shiley valve, revealed a large region of stasis downstream of the minor orifice, in both cases. Fluid remained in this region for more than 20 seconds. Due to the use of silicone rubber tubing between the diaphragm pump and the test chamber, the valves did not respond rapidly to changes in the pumping action, only opening and closing in a sluggish manner. This study is therefore unlikely to be representative of the type of behaviour of these valves in the heart, where the movement of the chamber walls will also assist valve opening. This poor operation of the Bjork-Shiley valve may also explain its poor performance coagulation-wise in this type of test.

## 8.2 The Belousov Zhabotinski Reaction<sup>221,222</sup>

When 150ml of 1M  $\text{H}_2\text{SO}_4$ , 0.175g of  $\text{Ce}(\text{NO}_3)_6(\text{NH}_4)_2$ , 4.292g of  $\text{CH}(\text{COOH})_2$  and 1.415g of  $\text{NaBrO}_3$  are mixed together<sup>223</sup>, the mixture turns yellow after a short delay (9-17 seconds). After a further delay of 3-4 minutes the mixture returns to a colourless state, oscillating from clear to yellow and back again



regularly thereafter with an initial period of oscillation of between 30 and 40 seconds, the period lengthening, and the intensity of colour reducing, with time.

It was thought that such an oscillating reaction would, if it were possible to predetermine the period of oscillation, provide a means of observing the fluid that had remained in the system for a given time interval. By arranging for the first oscillating period to coincide with the time period of interest, a continuous system might be operated so that the location of the yellow dye revealed where the fluid had been present in the system for the specified duration. Variations in location of the yellow dye during the pulsatile cycle should also reveal the changing pattern of stasis in different phases of this type of flow.

For such a system to work as desired, it is necessary that the oscillating reaction be chemically rather than diffusion controlled. Therefore, before attempting to vary the lag time and oscillatory period by changing the ingredient concentrations, it was considered prudent to investigate the effects of agitation on the system reported in the literature. The results for the first ten oscillations, at a variety of stirrer speeds, are reported in Table 7. Both the lag time and period of oscillation were found to vary with mixing conditions. Thus this experimental approach was abandoned.

**Table 7**  
**Period of Oscillation in Seconds of the Belousov Zhabotinski Reaction**  
**at Various Agitation Rates**

Period	Run 1 150 rpm	Run 2 250 rpm	Run 3 500 rpm	Run 4 700 rpm
First turns yellow	17	13	12	9
Turns clear again	230	227	199	171
2nd Oscillation Period	36	30	35	34
3rd Oscillation Period	43	40	36	36
4th Oscillation Period	42	48	31	34
5th Oscillation Period	38	42	35	36
6th Oscillation Period	48	37	33	41
7th Oscillation Period	40	40	40	37
8th Oscillation Period	44	39	29	41
9th Oscillation Period	44	48	35	32
10th Oscillation Period	46	48	41	48



## **Chapter 9. Theories on Shear-Induced Particle Migration**

One possible explanation for the observation that deposition occurs mainly in regions of stasis is that particles in the flow, capable of aggregating, diffuse preferentially into such regions. Regions of stasis are often bordered by more rapidly moving fluid, where the shear stresses are usually larger. This will be the case in the region downstream of Hladovec's net and in the wake of Vorhauer's objects (especially in Vorhauer's canine experiments where the objects formed a relatively large obstruction to the flow). If the forces on particles in such a shear field are of sufficient magnitude, and are directed towards the region of stasis, then it is conceivable that the concentration of particles in this region will be greater than that in the bulk fluid. If, also, the second stage of the clotting reaction is second order, involving binary collisions of aggregating particles (as suggested by Payens<sup>194</sup>), then a higher local particle concentration would lead to a greater aggregation rate, and thus a preferential site for clotting. Indeed, such an explanation may account for the fact that coagulum deposits downstream of Hladovec's net with both milk and blood, in spite of the absence of effective stasis (all the fluid having been in the recycle system for the same length of time). In this chapter, three forces that might possibly be thought to lead to preferential diffusion into regions of stasis, e.g. behind the struts of Hladovec's net, will be considered to ascertain their likely effects, if any, on particles in the flow. The three forces are: (1) those arising from the Magnus Effect, (2) Saffman's Forces and (3) forces due to free energy changes of particles distorted by the flow.

### **9.1 An extension of the Magnus Effect**

If the following assumptions are made, then the expected influence of the Magnus effect on particles in the flow downstream of Hladovec's net may be calculated:-

- i) The fluid after passing through the grid loses its momentum rapidly, so that within the cuvette it may be considered to be at rest. However, the particles maintain their momentum for a short distance downstream of the net.
- ii) The presence of the shear field within the grid causes the particles to spin around an axis, perpendicular to the flow and tangential to the fluid boundary, such that the axial velocity of the surface of the particle is at all points equal to that of the fluid with which it is in contact. The particles continue to spin at this rate for a short distance downstream of the net.
- iii) The velocity profile within the grid is approximately parabolic.



The force due to the Magnus effect,  $F_M$ , is defined in terms of the circulation of the particle,  $\Gamma$ , and its velocity relative to that of the fluid (which in the case of a stationary fluid will be the particle velocity,  $V_p$ ). By the Kutta – Jowkowski Equation:-

$$F_M = \rho \cdot V_p \cdot \Gamma \cdot D,$$

where  $D$  is the diameter of the particle and  $\rho$  is the density of the fluid.

By assumption (ii),

$$\Gamma = 0.5\pi \cdot D^2 \cdot dV_f/dy,$$

and by assumptions (i) and (iii),

$$V_p = V_f = ((a^2 - r^2) / 2r) \cdot dV_f/dy$$

and

$$dV_f/dy = 4V_{fm} \cdot r/a^2,$$

where  $V_f$  is the fluid velocity within the net,  $V_{fm}$  is the mean fluid velocity,  $a$  is half the mesh spacing and  $r$  is the radial displacement of the particle from the centreline of a hole in the mesh.

Thus,

$$F_M = 4\pi \cdot \rho \cdot D^3 \cdot V_{fm}^2 \cdot (a^2 - r^2) \cdot r/a^4.$$

If it is also assumed that the force acting against particle movement is given by Stokes Law, then the terminal radial velocity of the particle in the 'r' direction,  $V_{pr}$ , will be given by:

$$V_{pr} = F_M / (3\pi \cdot \mu \cdot D).$$

Assuming that the particle reaches its terminal radial velocity almost instantaneously, and that the axial component of its velocity remains constant, it is possible to estimate the distance downstream where the particle will pass into the shadow of the mesh struts, i.e. the longitudinal distance,  $x$ , travelled before the particle has travelled a radial distance of  $(a - r)$ .

To a first approximation,

$$x = (a - r) \cdot V_p / V_{pr}.$$

For the mesh in Hladovec's experiment  $a = 0.125\text{mm}$  and  $V_{fm} = 0.8\text{m/s}$ . The density,  $\rho$ , of blood is  $1025\text{kg/m}^3$  and its viscosity,  $\mu$ , is  $0.004\text{kg/m/s}$ . The diameter of red cells is approximately  $6\mu\text{m}$  and that of platelets is of the order of  $1\mu\text{m}$ . Considering the motion of both platelets and red blood cells at the radial displacement where  $F_M$  is a maximum (i.e.  $r = a / \sqrt{3}$ ) and at the extremity of their respective exclusion layers next to the wall (i.e.  $r = 0.124\text{mm}$  for platelets and  $r = 0.121\text{mm}$  for red blood cells), the values of  $F_M$ ,  $V_p$ ,  $V_{pr}$  and  $x$  are as follows:-



- a) For platelets at  $r = 0.072\text{mm}$ ,  $F_M = 2.5 \times 10^{-11}\text{N}$ ,  $V_p = 1.1\text{m/s}$ ,  $V_{pr} = 0.6\text{mm/s}$  and  $x = 95\text{mm}$ .
- b) For platelets at  $r = 0.124\text{mm}$ ,  $F_M = 10^{-12}\text{N}$ ,  $V_p = 25\text{mm/s}$ ,  $V_{pr} = 0.025\text{mm/s}$  and  $x = 1\text{mm}$ .
- c) For red cells at  $r = 0.072\text{mm}$ ,  $F_M = 5.5 \times 10^{-9}\text{N}$ ,  $V_p = 1.1\text{m/s}$ ,  $V_{pr} = 24\text{mm/s}$  and  $x = 2\text{mm}$ .
- d) For red cells at  $r = 0.121\text{mm}$ ,  $F_M = 9 \times 10^{-10}\text{N}$ ,  $V_p = 0.1\text{m/s}$ ,  $V_{pr} = 4\text{mm/s}$  and  $x = 0.1\text{mm}$ .

The distance downstream where platelets might be expected to cross over from the fast-moving fluid into the vortex region behind the mesh strut is too great, even for a particle starting  $1\mu\text{m}$  from the wall, to account for enhanced platelet transport into this region. With red blood cells such transport may be possible. However, the assumptions on which this analysis is based are extreme, being chosen to give an upper limit on the magnitude of the forces arising from the spinning of particles. The fluid motion immediately downstream of the grid is more likely to be identical to that within the grid rather than being stationary. Therefore it is worth investigating the forces on particles travelling within a shear-field, rather than downstream of one.

## 9.2 Saffman Forces

If one considers the forces on particles downstream of the grid arising from the presence of a shear-field in this region, it is possible to apply Saffman's theory for the forces on particles due to a shear-field<sup>116</sup>. If the velocity profile within the grid is again assumed to be parabolic, then the shearing rate will rise linearly with radial distance from the centreline of the flow channel through the grid. Two further assumptions will be made:-

- i) The shear field remains almost intact for a short distance downstream of the net.
- ii) The particles lose their momentum more slowly than the fluid.

Based on these assumptions, a reduction of 10% in the fluid velocity with negligible change in the longitudinal velocity of the particles will be considered. (The 10% reduction in shearing rates is accounted for in the analysis below.)

According to Saffman, the force,  $F_S$ , on a particle travelling in a shear-field is given by

$$F_S = 20.3 (V_f - V_p) \cdot D^2 \cdot \mu \cdot \sqrt{(dV_f/dy)/\nu}$$

Using the substitution introduced in the previous section for  $dV_f/dy$ , and rearranging,

$$F_S = 40.6 (V_f - V_p) \cdot D^2 \cdot \sqrt{(V_{fm} \cdot \rho \cdot \mu \cdot r)/a}$$



Assuming, as before, that Stokes Law applies:-

- a) For platelets at  $r = 0.072\text{mm}$ ,  $F_S = 5.5 \times 10^{-10}\text{N}$ ,  $V_{pr} = 13\text{mm/s}$  and  $x = 4.5\text{mm}$ .
- b) For platelets at  $r = 0.124\text{mm}$ ,  $F_S = 1.5 \times 10^{-11}\text{N}$ ,  $V_{pr} = 0.38\text{mm/s}$  and  $x = 0.07\text{mm}$ .
- c) For red cells at  $r = 0.072\text{mm}$ ,  $F_S = 2 \times 10^{-8}\text{N}$ ,  $V_{pr} = 90\text{mm/s}$  and  $x = 0.05\text{mm}$ .
- d) For red cells at  $r = 0.121\text{mm}$ ,  $F_S = 2 \times 10^{-9}\text{N}$ ,  $V_{pr} = 10\text{mm/s}$  and  $x = 0.04\text{mm}$ .

These values for  $x$  are an order of magnitude lower than those derived using the Magnus effect. The physical situation is also more plausible, since it does not necessitate the fluid coming to rest immediately downstream of the net. These Saffman forces, however, are likely to have the opposite effect on particles entering the net where the particles will tend to lag behind the fluid. At the entrance to the net these forces would be expected to cause the particles to migrate towards the centre of the flow channel, leaving a particle-depleted region near the grid surface. However, red cells, even near the centre of the flow channel, can migrate into the region behind the struts within  $50\mu\text{m}$  of the trailing edge of the net.

Whilst these forces may lead to a higher concentration of red cells (or indeed particles of a size equal to, or greater than, that of red cells) in the wake region, they will not account for an increase in platelet concentration, nor an increase in coagulating proteins, in this region. It is possible, however, that platelet aggregates of a sufficiently large size, already formed within the flow, may migrate preferentially into this region, or that, if the red cells assist platelet transport, as has been suggested (in a different context) by Forstrum<sup>158</sup>, then this movement of red cells may enhance platelet diffusion into this region.

### 9.3 Polymer Migration due to Shear-Induced Distortion

Dutta and Mashelkar<sup>115</sup> have suggested that, in dilute polymer solutions, polymer molecules may migrate from regions of high shear stress to those where the shear stress is low. High shear, it is postulated, leads to an elongation of the polymer molecules and their alignment in the direction of flow. This alignment and stretching creates spatial free energy differences, which in turn induces concentration gradients, resulting in the migration of macromolecules into regions of lower stress.

With both blood and milk it is possible that large proteins, and indeed formed elements, might be susceptible to a similar migration mechanism. However, it is expected that the forces involved would be insufficient to cause large concentration gradients to occur, though such a mechanism may lead to the preferential diffusion of larger molecules, whose susceptibility to stretching



will be greater, into low shear regions. Thus, partially coagulated proteins might be expected to preferentially enter such a region.

All of the work reported by Dutta has been carried out in capillary tubes, where the shear stresses are high and the migration distances low. It is thus prudent to enquire whether free energy changes would lead to perceptible migration in a large scale system (32mm diameter). Two students, F. Dickson and J. Russell, have carried out such an investigation using 2%wt. Carboxyl-modified Polyacrylamide in water. Their flow system comprised an 11mm diameter sharp edged orifice in a 32mm i.d. pipe, a flow straightener being incorporated 450mm upstream of the orifice. The polymer solution was allowed to flow, under the influence of gravity, at a flowrate of 5.5l/min through the test section. Samples of solution were taken isokinetically, through a 1.5mm i.d. sampling needle aligned with, and facing upstream into, the flow. Titrimetric analysis of samples taken from the vortex downstream of the orifice (low shear) and at the edge of the fluid jet, in the region of the vena contracta (high shear), revealed a 1 or 2% difference in the mean polymer concentrations measured in these regions, the concentration in the low shear region being consistently the higher. However, this variation was less than the experimental error (5 -10%), and the results are therefore inconclusive. This migration, therefore, whilst it may occur to a small extent in such a flow regime, is not expected to cause a significant spatial concentration variation.

Whilst none of these forces (with the possible exception of Saffman's forces) is expected to lead to a significant increase in particle concentration in a region of low shear, it is known that aggregation of large particles in such a region does actually occur. Goldsmith and Karino<sup>131</sup> have observed the behaviour of red blood cells and platelets in the region downstream of a concentric sudden expansion of a 150 $\mu$ m into a 500 $\mu$ m bore glass tube. Their experiments were carried out in steady flow. Red blood cells and aggregated platelets were observed to become trapped in the vortex. Thus, irrespective of the mechanism for the trapping of large particles in this vortex region, such entrapment does indeed occur in steady flow. It remains to be seen whether such entrapment also occurs in pulsatile flow, or indeed whether such entrapment is responsible for preferential deposition in this region.



## Chapter 10. Discussion

The aim of this chapter is that of uniting the various aspects of the research presented in the previous chapters. Much of this discussion, therefore, will appear similar to that already presented, although individual points may be covered in less depth. Where this is the case, reference will be made to the previous discussion.

### 10.1 Length of Test Chamber

The Lee White clotting time for the milk mixture lay between 30 and 45 seconds at the operating temperature used in these experiments. The original test chamber was designed such that the entrance region was 30 pipe diameters long and the fluid reached the drain before 30 seconds had elapsed. The residence time of the activated milk prior to encountering the axisymmetric test objects was  $21 \pm 6$  seconds. With this system, the deposit of clot totally encased the object, suggesting that deposition might be due to suspended particles, already formed within the milk, impinging on the object. A shorter duration experiment, in which the initial deposition of clot was observed, revealed that the build up of clot started upstream of the object, followed soon after by deposition in the wake region. This coagulum was particulate in nature, apparently being due to the impingement of  $\sim 0.5$ mm diameter particles.

This type of behaviour is not found with thrombosis around objects placed in the bloodstream and, indeed, is not observed in the deposition patterns of milk clot in Lewis's experiments.

A test chamber with no entrance region, in which the milk reaching the object had a residence time of  $2 \pm 1$  seconds, provided a different type of deposit. Here coagulum formed preferentially in the wake region of the test bodies, (as with Vorhauer's experiments with blood), indicating a preferential formation of coagulum at such sites, similar in many respects to that found with blood. A medium length test chamber gave an identical clotting pattern but with less milk being required to form a given weight of clot (See Figs. 23, 24a & 26a). In this medium length chamber the residence time of the fluid in the entrance region is  $9 \pm 3$  seconds. Thus it would appear that the length of the entrance region does not affect the clotting pattern so long as it is below a certain critical length.

This localised, flow dependent, deposition appears to occur in all cases where the maximum residence time of activated milk prior to encountering the test piece is less than half the Lee White clotting time. A possible explanation for this difference in behaviour, between coagulation in the long chamber and

that in the shorter chambers, is yielded by the modified Lee White test. In this test, agitation of the mixture when particles first start to appear (at about half of the Lee White clotting time) results in a preferential deposit of coagulum at the wall of the test tube. Agitation, however, does not appear to alter the length of the lag phase of the reaction. This critical state for wall deposition would occur after 15 - 20 seconds at the temperature used in both the long and medium test chambers and after about 12 seconds in the short test chamber experiments. Thus in the long test chamber the bulk fluid would have reached this state, so that particles would already be formed in the fluid reaching the test object, leading to an even covering of coagulum at all points where the fluid impinges on the surface of the test body. In the short and medium test chambers, however, only fluid trapped in regions of stasis would reach this state whilst still in the vicinity of the solid body.

## **10.2 Milk Coagulation Around Axisymmetric Test Bodies**

Two sets of experiments were conducted in the medium length chamber; one in steady flow, the other in pulsatile flow. (A more detailed discussion of the nature of the deposition observed is given in Section 6.1.4.). The coagulum deposited on the upstream face of each object varies more in quantity from run to run, is generally more randomly spread, and has a more coarse-grained spongy appearance than that downstream. This deposit is therefore thought to be adventitious, being caused by vapour bubbles adhering to the object, by surface irregularities, or by impinging particles of clot dislodged from the test chamber wall further upstream.

The nature of the downstream coagulum, on the other hand, indicated a more orderly deposition pattern. This pattern was markedly different for the two flow regimes.

In steady flow, deposition occurred preferentially in the wake of the test bodies. In experiments with dye injected at the same position as is rennet in the milk tests, colour was found to persist in the wake of all the objects for periods in excess of 20 seconds. The size of wake region and the maximum residence time of the fluid did not appear to influence the size of clot deposited. A possible explanation of this behaviour is that deposition of milk clot from milk (having reached a critical clotting state) will modify the shape and size of the wake region. Dye experiments with unfouled test bodies will only show the initial size of the wake regions and may thus not yield a good indication of the extent of coagulation after a prolonged period.

In pulsatile flow the downstream face of both the cone and the disc remained almost devoid of deposit. Dye injection experiments revealed that any vortices formed during systole were shed during diastole for these test



objects. Thus the fluid surrounding these objects would not reach a coagulable state. With the tear-drop and sphere, on the other hand, an azimuthal band of clot occurs on the downstream of each of these test bodies at the point where separation of the forward flow from the body's surface might be expected to occur. Dye experiments indicate that a pocket of fluid is trapped downstream of these objects, oscillating to and fro with the pulsatile motion. The region where most coagulum deposits is at the upstream extremity of this motion where the pocket of fluid impinges on the surface and where the fluid remains for most of the diastolic period. The presence of stasis is thus apparently a necessary factor in the preferential formation of a band of clot in this region, whether or not other factors, such as flow impingement or surface contact time, are also of critical importance. Stasis alone is unlikely to be the cause of this thicker band of clot, as the dye experiments show that the trapped pocket of fluid moves downstream during systole, in contact with the surface of the test body, whereas only a thin layer of clot forms in this region.

In the context above, surface contact time is essentially different from stasis. Pockets of fluid in the wake of the test body may have been present for periods in excess of the time necessary for the coagulation reaction to have reached the stage where deposition is possible. However, these pockets of fluid may spend relatively little time actually in contact with the surface of the object, and thus only lead to a relatively low deposition rate. Indeed it is possible (as suggested by Goldsmith and Karino<sup>131</sup>) that larger particles will migrate to the centre of a vortex region, and thus rarely come into contact with the solid surface. Therefore, whilst the presence of residence times of sufficient length are expected to lead to deposition of clot, the rate of deposition may be dependent on the length of time that the activated fluid spends in intimate contact with the surface, and indeed on its velocity of travel across the surface.

### **10.3 Comparison With Vorhauer's Results**

Vorhauer, using test objects implanted in the canine descending aorta found that thrombus deposited in the wake of the test bodies, and suggested that the amount of thrombus depended on both the size and turbulence intensity in the wake region. Whilst Vorhauer demonstrated in a second set of experiments that turbulence has an influence on the quantity of thrombus deposited, it is by no means certain whether either the size of, or the turbulence intensity in, the wake region, or indeed both, are responsible for the quantities of coagulum deposited. In order of increasing wake thrombus weight the objects were tear-drop (0.0301g), sphere (0.0658g), disc (0.0831g), downstream apex cone (0.0949g) and upstream apex cone (0.1478g).

For the milk flow experiments reported here the ordering in steady flow was sphere (0.043g), disc (0.071g), tear-drop (0.080g) and upstream apex cone (0.095g), and in pulsatile flow upstream apex cone (0.006g), disc (0.027g), sphere (0.073g), downstream apex cone (0.074g [thought to have been carried over from the upstream face of the object]) and the tear-drop (0.200g). Neither of these sets of data agree fully with that of Vorhauer. The closest similarity appears to be with the steady flow experiments reported here, with the sole exception of the tear-drop.

In Vorhauer's experiments the test objects occluded almost half of the canine aorta giving much higher shear stresses around the edges of the test bodies than in these milk experiments where only one tenth of the flow channel was occluded. This would have a marked influence on the size of, and turbulence intensity in, the wake region and would also preclude deposition on the lateral faces of the objects. Since the aorta is flexible the test bodies may have even made contact with the endothelium, dislodging any thrombus that may have formed in this region.

Due to the relatively large degree of occlusion and the flexibility of the test chamber walls, the flow in Vorhauer's experiments is likely to have a large steady component with only a small oscillating component superimposed on it. The high degree of occlusion would lead to high pressure drops across the length of the test bodies, which, coupled with the compliance of the muscular aorta walls and the alternative route for blood flow via the ascending thoracic aorta, would readily damp out the oscillatory component of the flow. In the rigid milk test chamber the pulsatile flow encountered resembles that found at the exit of the heart, i.e. a strong pulse of fluid during systole with an almost stagnant period during diastole. Thus the flow in Vorhauer's experiments most closely resembles steady flow.

It is not surprising, therefore, that the milk experiments in steady flow give similar clotting patterns to those of Vorhauer. The difference in position of the tear-drop in relation to the clotting propensity may be due to one of two factors: A change in the nature of the wake due to the proximity of the test chamber walls, or a slight difference in the shape of the tail of the tear-drop, leading to boundary layer separation from the surface in the case of the milk experiments (but not Vorhauer's experiments). The proximity of the test chamber walls will help to reduce the possibility of boundary layer separation in the case of Vorhauer's experiments.



#### 10.4 Bjork Shiley Valve in Steady and Pulsatile Flow

The Bjork-Shiley valve was tested in the long test section (See Section 6.2 for a full discussion.). In all but one experiment the conditions for deposition were the same as for the axisymmetric test bodies in the long test section, i.e. the bulk fluid had reached the critical stage for coagulation on arrival at the valve. The type of deposition encountered is therefore reminiscent of that occurring in a snow storm. The minor orifice becomes blocked by a ramp of clot on both its upstream and downstream sides whilst the major orifice remains clear of clot due to the high flowrates in this region. In dye injection experiments, residence times in excess of 20 seconds are found in the wake of this valve under all the flow conditions studied.

In the pulsatile flow experiment at 2l/min the downstream ramp was not flat in lateral cross-section, having a central ridge and rising again towards the walls. Dye experiments show a strong secondary flow in this wake region with fluid returning along the wall and flowing towards the centre of the disc as it mixes with the flow through the minor orifice before progressing downstream once more. This secondary flow may serve to reduce the deposition of clot along the lateral edges of the disc, whilst the stagnation point formed on the centre-line of the disc might promote deposition. This central ramp of clot does not appear in either the pulsatile flow experiments at 1.5l/min or the 3l/min steady flow. The absence of this ridge in steady flow may be explained in terms of the absence of this secondary flow pattern but in the case of the lower flow rate pulsatile flow its absence is anomalous. One possible explanation is that the valve stopped moving in this test at an earlier stage than it did at the higher flowrate so that this secondary flow may not have persisted for so long.

During one of the steady flow experiments the rennet injection system developed a leak. An attempt to rectify the problem was not fully successful but the experiment was continued, the rennet concentration being then less than usual. An interesting result emerged. The milk clot formed a smooth thin layer on the disc with a thicker clot forming at the base of the struts. Since the enzyme concentration would be lower due to the leak the Lee White clotting time would be correspondingly longer. Thus in this test, as with the medium length test chamber experiments using the axisymmetric bodies, the deposit of coagulum may be expected to depend on the presence of regions of stasis close to the valve. This type of milk curd deposition in the region of the Bjork-Shiley valve was, in this case, quite similar to that observed by Lewis in his experimental heart chamber.

## 10.5 Limitations of the Testing Technique in this Test Chamber

In many of these tests it was not appreciated that the milk mixture should have been activated for less than half the Lee White clotting time prior to encountering the test object. This can be achieved by varying the concentrations of the injected components or by varying the milk temperature, though care must be taken in ensuring that the milk curd formed in these altered circumstances is sufficiently adherent to the test object.

A limitation inherent in the apparatus is the inevitable deposition of milk curd in the boundary layer region at the test chamber wall. This region is essentially a region of stasis in which the milk mixture reaches a hypercoagulable state. Experiments in an empty 12.7mm i.d. tube reveal that the first signs of coagulation occur a distance of 30cm from the mixing point. Assuming that a reaction time of 20 seconds is necessary before deposition will occur, the absence of clot in this region might suggest that the stratum of fluid closest to the wall in which the reaction is reaching this critical state is travelling at approximately 15mm/second. Using the Nikuradse-Prandtl equation for the Law of the Wall ( $U^+ = 2.5 \ln y^+ + 5.5$ , where  $U^+ = U \sqrt{(\rho/\tau_w)}$  and  $y^+ = y \sqrt{(\tau_w \rho)}/\mu$ ) and assuming that  $\tau_w \sim 5 \text{ N/m}^2$  (Calculated from an estimate of the friction factor) this fluid velocity would occur at a distance of  $4\mu\text{m}$  from the wall. Since the micelles are approximately  $0.3\mu\text{m}$  in diameter, this apparent absence of deposition from fluid travelling slower than 15mm/second cannot be explained simply in terms of a micelle exclusion layer at the wall. Possibly (as indicated in Section 6.3) diffusion of rennet into the boundary layer, or the thickness of the wall layer containing milk at the critical stage for deposition, are also important.

Since coagulation occurs in this boundary layer at the wall, attempts at determining preferential sites of thrombus on wall mounted objects are difficult, if not impossible, to perform. This difficulty is heightened by the tendency for the boundary layer wall clot to become dislodged from the wall on dismantling the test section. Experiments reproducing Gott's vena caval rings, with and without orifices, or Vorhauer's second set of tests measuring thrombosis on the mounting ring were thus impractical with the present apparatus. Some evidence of a thinning of the wall clot at a distance of 0.5" downstream of the disc and cone were indeed observed, but the degree of thinning and the extent of the thinner region were unable to be determined.

A further limitation of the technique, in some senses associated with both of the aforementioned experimental shortcomings, is that rennet is added to the bulk milk mixture. In thrombosis the release of clotting agents into the blood is likely to be highly localised, occurring in areas of high shear or where platelets have adhered to a solid surface. Thus dilution of these clotting



agents (if present only in small quantities) with the bulk fluid can prevent the clotting reaction from proceeding to a stage where fibrin formation will occur, the activated enzymes being deactivated by other reactions in the blood. In the milk tests, on the other hand, rennet is injected into the bulk flow so that the whole milk stream will clot after a known time. No processes exist in the milk or the test rig for deactivating the rennet.

## **10.6 Stasis as an Explanation for Milk Clot Deposition**

In the experiments conducted with the medium length test chamber, adherent coagulation is only found to occur in regions of stasis. Thus milk curd is deposited in the wake of all the objects in steady flow, and only downstream of those objects in pulsatile flow where a vortex is found to be at least partially trapped during the pulsatile cycle. The boundary layer where stasis is also present is likewise a site of milk clot deposition.

Lewis<sup>80</sup> repeated the experiments of Petschek<sup>66</sup> and Hladovec<sup>64</sup> using a similar milk mixture. The behaviour of the milk in these experiments was remarkably similar to that of blood. In a replica of Petschek's stagnation point flow chamber, Lewis observed wedge-shaped milk clots to deposit, apparently initiated by small projections on the cover-slide. Here the milk mixture, at least in the high shear chamber where wedge-thrombi are observed, remains for less than half the Lee White clotting time. It would appear plausible that the localised region of stasis downstream of such a surface imperfection may result in a small deposit at the surface. This coagulum, once deposited, would serve to increase the localised stasis in its wake leading to further growth of the milk clot on its downstream face. Since milk clot will deposit even within the boundary layer at the wall of the test chamber in the replica of Vorhauer's experiments, it seems likely that even highly localised regions of stasis may allow the reaction to proceed to a critical point for deposition.

In Hladovec's net experiment clot appears in the wake of the nylon mesh where stasis would be expected. However, in the recirculating milk flow system, the residence time effects of such stasis are eliminated in that all the fluid, whether in the wake of the net or not, has been activated by the enzyme, Chymosin, for the same length of time. This is not the case in our single-pass milk test rig, where the bulk fluid reaches the drain before the induction period is complete. A further flow property, other than the extended residence time, is therefore required to account for the preferential formation of clot in the wake of the net.

In chapter 9 a discussion is presented of the possible forces acting on particles suspended in the fluid which might lead to a local build up in platelet or red blood cell concentration downstream of Hladovec's net. However, as

concluded in that chapter, whilst such processes may aid diffusion of particles into such a region, leading to entrapment of sufficiently large particles (as observed by Goldsmith<sup>131</sup>), it is unlikely that such forces will lead to a higher localised concentration of activated coagulation enzymes, except in so far as these are released from, or contained in, the surface of platelet aggregates or red blood cells. In milk the forces on micelles will be as small as those on platelets, if not smaller, so that it seems unreasonable to suppose that a significantly larger proportion of micelles will be present in this region. Since milk and blood appear to form microscopically similar deposits, in both cases preferentially downstream of the net, it would therefore seem prudent to suggest an explanation for this coagulation that might equally apply for both fluids. Either surface contact time or agitation in the region of the solid surface would thus appear to be the more likely explanations for this similar clotting behaviour of blood and milk

Lewis also observed coagulation of a similar milk solution in the vicinity of the Bjork-Shiley, Starr-Edwards and Edinburgh-Pivoting-Disc heart valves using a mock heart flow chamber described in his thesis<sup>80</sup>. He found that with each of these valves the milk clots occurred in similar locations to thrombus found in clinical practice. In the case of the Bjork-Shiley valve coagulation occurred principally around the struts, with a uniform coating of the disc if the temperature and rennet concentration were higher (possibly in a process similar to the 'snowstorm' effect postulated earlier). With the Starr-Edwards valve coagulation occurred in the region of the sewing ring and around the struts, with no coagulum adhering to the ball. This is similar to thrombus deposition in clinical practice. Using the Edinburgh valve an even covering of the disc was observed (in conditions similar to those where an even covering of the Bjork-Shiley disc occurred), with a slightly thicker clot on the downstream trailing edge. In animal tests this valve was found to remain substantially free of thrombus<sup>9,216</sup>.

With the Starr-Edwards valve the sewing ring has a restrictive orifice, to provide a seat for the ball on flow reversal. This orifice may have a small region of stasis on its upstream side and a region of stasis downstream. Thus coagulation in this region is consistent with stasis being a requirement for a coagulation site. The struts of both the Starr-Edwards and Bjork-Shiley valves may produce some stasis in their wakes leading to deposition in this region. As in the case of Hladovec's net, this is likely to be extremely localised, which may account for the fact that many Starr-Edwards valves removed during autopsy have thrombus solely in the region of the valve sewing ring. With the aerofoil-shaped Edinburgh valve the downstream trailing edge again may be the site of a localised region of stasis, though the behaviour of this stasis on flow



reversal would have to be examined further.

### **10.7 Role of Stasis in other Blood Clotting Experiments**

In Gott's experiments<sup>62</sup>, the fairing of the upstream face of the orifice, which can eliminate thrombus, would remove a region of stasis and separated flow from this area, but further investigation would be necessary to analyse whether stasis is occurring downstream of the orifice, where no thrombus is reported to be found. Blackshear<sup>63</sup>, in his analysis of the effects of fairing of the orifice and of the position of the orifice within the vena caval ring, indeed claims that stasis, encountered in the vortex downstream of the orifice, coupled with a release of activated reagents into this region, could account for the variations in clotting behaviour observed. As indicated earlier (Chapter 3) this explanation would only apply if thrombosis occurs predominantly to the downstream side of the orifice.

The turbulent shunts of Smith<sup>123</sup> and Stein<sup>124</sup> also contain orifices. Whilst Smith and Stein both demonstrated using dye experiments that no region of prolonged stasis was present downstream of the orifice in their turbulent flow shunts, it is possible that even short-term stasis, together with a greatly increased level of interaction between the fluid and the inner surface of the shunt, will lead to coagulation. That more thrombus forms in Smith's rather than Stein's experiments (4.9g as opposed to 0.2g for the turbulent shunts), may be attributed to two factors:-

- i) In Smith's experiments the experimental duration was longer, the turbulent Reynolds number being allowed to drop from 2200 to about 600 (Stein's experiments lasted for only 7 minutes to ensure that the Reynolds number remained constant).
- ii) In Smith's experiments the flowrate was allowed to drop. Whilst the flow was always turbulent, this will have increased the scale of the residence times within the shunt, possibly giving a greater clotting propensity.

Generalised stasis, it would appear, is not a satisfactory explanation for the clotting in these shunts.

In Leonard's shunt<sup>125</sup>, incorporating a square-edged circumferential recess, clot was found only to occur in the regions of stasis in the corners of this groove. Both prolonged surface contact time and flow impingement on a surface will occur, too, in this region.

With Davila's pins<sup>119</sup> of various cross-section, the round and triangular pins became enveloped in clot, whereas an elliptical pin only developed a streamer of clot on its downstream side. This is in agreement with the size of wake regions expected with these shapes of pins.

## **Chapter 11. Conclusions and Recommendations for Future Work**

### **11.1 Conclusions**

- i) A milk mixture containing rennet and  $\text{CaCl}_2$  can be used to test for flow related coagulation, so long as no particulate matter is visible in the milk mixture entering the flow situation under test. The milk mixture, introduced to the test object prior to half of the Lee White clotting time elapsing, resulted in localised clot deposition on the test body, whereas that introduced after a greater duration resulted in a generalised covering of the test body with clot.
- ii) Deposition of milk curd in the shorter test chambers occurs only in areas of stasis, i.e. in the wake of the test objects in steady flow, and in specific regions where a trapped vortex exists in pulsatile flow. The boundary layer at the wall of the tubular test chamber is such a region of stasis.
- iii) Turbulence of the milk mixture in the presence of a solid surface, at the stage of the reaction where insoluble particles are first starting to appear, leads to a preferential adhesion of the coagulum to the surface. This is demonstrated by the use of a modified Lee White test.
- iv) Stasis is not the sole criterion for the preferential adhesion of coagulum in particular regions. This is borne out by the absence of a uniform clot along the full extent of oscillation of the trapped vortex, in the case of the tear-drop and sphere in pulsatile flow. The modified Lee White test leads to the suggestion that turbulence, or impingement of fluid on the surface might be concomitant conditions. Another factor may be the time spent in intimate contact with the surface of pockets of fluid that have reached the critical stage for deposition. In the case of the tear-drop and sphere the thicker band of clot appears where the trapped vortex remains for most of the diastolic period. This more static behaviour of the fluid during diastole may also explain the earlier observations of the apparent upstream propagation of the wedge shaped wall clot.
- v) Areas of stasis need not be large in order to serve as preferential clotting sites. Surface imperfections (Petschek), tapered orifices (Stein & Sabbah), and fine grids (Hladovec) or struts in the fluid stream, or indeed the



boundary layer at the wall of a uniform tube, are all suitable candidates for coagulation sites.

vi) Deposition of thrombus in Vorhauer's experiments differed both in quantity and quality from that of milk curd around the same shapes of objects. The differences in extent and pattern of deposition are thought to be due mainly to the difference in cross-sectional area of the objects relative to that of the flow channel. The flow in Vorhauer's experiments is thought to be more akin to steady flow than to the type of pulsatile flow obtaining in the experimental apparatus used with milk.

vii) Steady flow experiments are not necessarily good indicators of the regions where coagulation is most likely to occur *in vivo*. Areas of stasis in strongly pulsatile flow, such as that found in the heart, may be different from those found in steady flow, vortex shedding being more probable in pulsatile flow.

viii) The milk experiments differ from blood clotting situations in one very important respect. In blood the release of clotting reagents is localised, occurring in regions of high shear and next to a foreign surface, though there is a possibility of a build-up in the bulk concentration of such reagents, depending on the efficiency of the body in destroying such chemicals and on the liver in its role as a purge. Whilst this local nature of activation is not important in the heart, where swirling of the fluid will lead to bulk mixing, it is likely to be important with Vorhauer's experiments in the canine aorta. In these milk experiments, on the other hand, enzyme, of a concentration great enough to cause bulk coagulation of the milk, is injected into the bulk flow so that the fluid as a whole will coagulate given sufficient time.

## **11.2 Recommendations for future work**

i) A more thorough and exhaustive investigation of the reproducibility of the milk tests reported herein is necessary before use of this apparatus for standard coagulation tests.

ii) The experiments on the Bjork-Shiley valve in steady and pulsatile flow should be repeated in the shorter test chamber, and under flow conditions ensuring the correct function of these valves.

iii) Analysis of coagulation around the axisymmetric test bodies should be carried out in a smaller diameter test chamber in pulsatile flow, with a

compliance chamber upstream of the test objects, so as to more faithfully mimic the flow in the canine descending aorta, encountered in Vorhauer's experiments.

iv) Vortex shedding in physiological pulsatile flows should be examined, with a view to designing a valve in which the residence time in the region of the valve is minimised.

v) An *in vitro* system should be designed for administering local injections of rennet in regions where activation of clotting factors is expected *in vivo*. This is not particularly important in the heart chamber where uniform mixing of such activated factors is likely to occur, but in the aorta or vena cava such local activation will possibly influence the regions of deposition to a marked extent.

vi) Whilst the Belousov-Zabotinski reaction was not pursued in this programme of work, its use as a tool for locating regions of stasis in pulsatile flow is not entirely ruled out. Further investigation of this oscillating reaction may well yield fruitful results.

vii) A means of eliminating the generalised deposition of clot on the wall of the test chamber is desirable. One possibility might be to arrange the test chamber in such a way that the fluid adjacent to the wall has a different composition from that in the bulk, i.e. by injection of a second fluid through an annular slot in the wall in a manner resembling a wetted wall column. Whilst such a system may be feasible in steady flow, it seems unlikely that a stable layer of fluid, of different composition from that in the bulk, could be maintained adjacent to the wall in pulsatile flow.



## **Appendix A**

### **Dimensions of Plate Heat Exchanger**

Plate width : 70mm  
Plate height : 578mm  
Plate thickness : 22swg (0.71mm)  
Plate spacing : 2.74mm  
Heat Transfer area / plate :  $0.025\text{m}^2$   
Number of heat transfer plates : 10  
Plate material : 316 Grade stainless steel  
Gasket material : Paracril  
Milk capacity : 0.3litres

### **Plate Arrangement**

6 parallel hot water channels alternating with 5 milk channels  
Milk flow countercurrent to Water flow  
25l/min water enters at  $45^{\circ}\text{C}$  and leaves at  $\sim 38^{\circ}\text{C}$ .  
Up to 5l/min milk enters at  $5\text{--}10^{\circ}\text{C}$  and leaves at  $35\text{--}42^{\circ}\text{C}$ .  
Pressure drop across exchanger (water side) : 0.1bar  
Pressure drop across exchanger (milk side) : 0.01bar

## **Appendix B**

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## A STUDY OF THE PERFORMANCE OF A STATIC MIXER IN PULSATILE FLOW

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### Summary

It is required to provide a means by which two reagents, supplied at equal rates by metering pumps, can be uniformly mixed on-line with a pulsatile stream of milk having fifty times their combined flow rates, at the entry to a rig for evaluating the thrombogenic tendency of prosthetic heart valves. Results are reported for the uniformity of composition obtained under various conditions of operation in trial experiments, with model fluids, using a particular type of commercial static mixer proposed for this application.

The uniformity of mixing depends on three factors:

1. The configuration of the injection system.
2. The pulse amplitude, shape and frequency of the main flow, both absolutely and relative to the pulsatile characteristics of the injected stream.
3. The performance of the static mixer, as measured in steady flow experiments.

In the experiments reported here, injections are made through a nozzle at the axis of the main fluid flow. The reagent injection pumps and the main flow pulsatile pump (whose flow characteristics can be made to resemble those of the human heart) are independent, i.e., not phase or frequency linked. We have observed the performance of a Chemineer Kenics KMS static mixer as a function of the frequency and pulse shape of the main flow in these circumstances, and have compared these pulsatile results with those obtained in steady flow. The method used has been to inject electrolyte into water, making measurements of the resulting conductivity immediately downstream of the static mixer.

A dimensional analysis enabled an assessment to be made of the importance of the various operating parameters. The best mixing was achieved in pulsatile flow with a dimensional pulse number greater than two and the frequencies and phase of the main fluid flow and injection flow being matched, the Reynolds Number and main fluid pulse shape having limited effects.

# NOMENCLATURE

$C_{max}, C_{min}, \bar{C}$	=	Maximum, minimum and average salt concentration	kmol/m <sup>3</sup>
$D$	=	Static mixer internal diameter	m
$f$	=	injection pulse frequency	Hz
$F$	=	main pulse frequency	Hz
$f_r$	=	required resolution frequency	Hz
$N_c$	=	dilution number = $\dot{v}/\dot{V}$	-
$N_f$	=	frequency number = $f/F$	-
$N_{ld}$	=	static mixer length/diameter ratio = $4V_{sm}/\pi D^3$	-
$N_p$	=	pulse number = $V_{sm}f/\dot{V}$	-
$N_r$	=	resolution number = $V_{cc} f_r/\dot{V}$	-
$N_s$	=	static mixer shape factor	-
$Re$	=	Reynolds number = $4\dot{V}_p/\eta\pi D$	-
$\dot{v}$	=	volumetric flowrate of injection fluid	m <sup>3</sup> /s
$\dot{V}$	=	volumetric flowrate of main fluid	m <sup>3</sup> /s
$V_{cc}$	=	conductivity cell volume	m <sup>3</sup>
$V_{sm}$	=	static mixer volume	m <sup>3</sup>
$w$	=	injection pulse shape factor	-
$W$	=	main fluid pulse shape factor = systolic/diastolic duration ratio	-
$\Delta$	=	degree of segregation = $(C_{max} - C_{min})/2\bar{C}$	-
$\eta$	=	dynamic viscosity of water	Ns/m <sup>2</sup>
$\rho$	=	density of water	kg/m <sup>3</sup>
$\phi$	=	phase angle between injection and main flow pulses	-
$\psi$	=	general function	-



## 1. INTRODUCTION

In studies of the causes of blood clotting (thrombosis) in artificial heart valves it has been shown, by Lewis (Ref. 1), that a freshly-prepared, homogeneous mixture of milk, rennet and calcium chloride, forms a clot at the same sites on currently used heart valves as those affected by thrombosis in clinical practice. Such a coagulable milk mixture can therefore be used in investigations of the causes of valve-related thrombosis and in evaluating the relative clotting propensities of artificial heart valves of different design.

To facilitate such investigations, an experimental apparatus is required capable of supplying to any heart valve under test a pulsating flow of milk containing 1% by volume each of rennet and saturated aqueous calcium chloride solutions. As the mixture is unstable, the two reagents must be introduced into the pulsating pumped flow of milk as soon as possible before entry to the valve under test. However, for valid tests of the influence of local flow characteristics at the valve on clotting tendency, the composition of the test mixture must be uniform both spatially (across the valve diameter) and temporally (at every phase of the pulsatile pumping cycle).

The experiments described here have been performed to investigate how well a commercial static mixer is able to satisfy these requirements for homogeneity in the kind of pulsating flow required. Electrolyte was injected into steady or pulsatile flows of pure water, the resulting conductivity being measured immediately downstream of the static mixer.

## 2. DIMENSIONAL ANALYSIS

The measured degree of segregation,  $\Delta$  [defined as  $(C_{\max} - C_{\min})/2\bar{C}$ ], was assumed to depend on the following operating variables:-

- The main fluid flowrate ( $\dot{V}$ ), frequency ( $F$ ), pulse shape ( $W$  = systolic : diastolic ratio), viscosity ( $\eta$ ) and density ( $\rho$ ).
- The injection fluid flowrate ( $\dot{v}$ ), frequency ( $f$ ), pulse shape ( $w$ ) and phase angle ( $\phi$ ).
- The static mixer volume ( $V_{sm}$ ), diameter ( $D$ ) and element type and arrangement denoted by a combined shape factor ( $N_s$ ).
- The conductivity cell volume ( $V_{cc}$ ).

Thus,  $\Delta = \psi (F, \dot{V}, W, \eta, \rho, f, \dot{v}, w, \phi, V_{sm}, D, N_s, V_{cc})$ .

The dimensionless segregation thus depends on thirteen variables in three dimensions including four dimensionless quantities, implying the need for six dimensionless combinations of the dimensional quantities. The groups chosen were:-

- The Frequency Number ( $N_f = f/F$ ) representing the number of injection pulses per main flow pulse.
- The Dilution Number ( $N_c = \dot{v}/\dot{V}$ ); the volume fraction of the injection flow relative to the main flow.
- The Pulse Number ( $N_p = V_{sm}f/\dot{V}$ ); the number of injection pulses per static mixer volume.
- The Reynolds Number ( $Re = 4\rho\dot{V}/\pi\eta D$ ).
- The Mixer Length/Diameter Ratio ( $N_{ld} = 4 V_{sm}/\pi D^3$ ).
- The Resolution Number ( $N_r = V_{cc} f_r/\dot{V}$ , where  $f_r$  is the desired sampling rate).

Therefore,  $\Delta = \psi (N_f, N_c, N_p, Re, N_{ld}, N_r, N_s, W, w, \phi)$

The same static mixer, model fluid, conductivity cell and injection pulse shape were used throughout, therefore  $V_{sm}$ ,  $D$ ,  $\rho$ ,  $\eta$ ,  $V_{cc}$ ,  $N_s$ ,  $N_{ld}$  and  $w$  remained constant. A constant dilution of  $N_c = 0.01$  was chosen to match that of the milk system.  $N_r$  represents the ratio of the sampling frequency to the volume of changeover frequency within the conductivity cell, which must be less than one to give the required sampling resolution. A sampling frequency  $f_r$  of 10 Hz was chosen to resolve events occurring every tenth of a pulse for the normal main flow frequency of 1 Hz, giving  $N_r < 1$  for these experiments.



### 3. EXPERIMENT

The effects of  $N_f$ ,  $N_p$ ,  $W$  and  $\phi$  on  $\Delta$  were investigated for pulsatile flow at four Reynolds Numbers (1040, 1640, 2230, 2530) and the effects of  $N_p$  and  $Re$  on  $\Delta$  were investigated for steady flow.

A schematic diagram of the apparatus is shown in Fig. 1. The Macleod positive displacement diaphragm pump [see Knight (Ref. 2)], whose frequency ( $20-150 \text{ min}^{-1}$ ), stroke volume (0-100 ml) and systolic-diastolic duration ratio (1:2.5 - 2:1) can be varied independently, pumped water at  $25^\circ\text{C}$  through a static mixer having six helical elements each 22.5 mm long and a 15.7 mm internal diameter, equal to that of the main flow pipe. A 5M aqueous NaCl solution was delivered along the main flow axis, via a 1 mm bore nozzle, by a diaphragm metering pump with variable frequency ( $15-100 \text{ min}^{-1}$ ) and stroke volume (0-0.6 ml). Two carbon electrodes (4 mm long x 7 mm wide) lying diametrically opposite each other, flush with the pipe wall, formed the conductivity cell with an Ultra-Violet Oscillograph recording the temporal variation of the conductivity and the pump phases.

### 4. RESULTS

The influence of  $N_p$  is dominant when  $N_p < 1$  in all instances. (Fig. 2).

4.1 Harmonic frequencies :  $N_f = 0.25, 0.5$  and  $1.0$ ;  $W = 1.0$ . (Fig. 2a)

- a)  $\phi$  is important (best mixing with  $\phi = 0$ ), though this importance decreases as  $N_p$  increases.
- b) For  $N_f = 0.5$  and  $1.0$  satisfactory mixing is obtained when  $N_p > 2.0$ , irrespective of  $\phi$ .
- c) For  $N_f = 0.25$  the effect of  $\phi$  is less though  $\Delta$  remains high at large  $N_p$  values

4.2 Non-harmonic frequencies :  $N_f = 0.75, 1.33$  (Compared with  $N_f = 1$ );  $W = 1.0$  (Fig. 2b).

- a) The effect of  $\phi$  is small, since phase matching is impossible for all pulses.
- b)  $\Delta$  remains relatively high, even at large  $N_p$  values.

4.3 Pulse shape variations :  $W = 0.4, 1.0$  and  $2.0$ ;  $N_f = 1$  (Fig. 2c)

- a) There is no observable influence of the main fluid pulse shape on  $\Delta$ .

4.4 Steady and pulsatile flows compared.

- a) In steady flow, increasing  $Re$  enhances the mixing quality.
- b) In pulsatile flow, changes in  $Re$  from 1040 - 2530 had no apparent effect on mixing quality.
- c) Pulsatile flow with  $N_f = 1$  and  $\phi = 0$  gave more uniform mixing than steady flow at similar values of  $Re$ .

### 5. CONCLUSIONS

- 1) The pulse number has a dominating effect, especially when less than one where a large spread of residence times would be necessary to provide good mixing.
- 2) The effect of the frequency number relies both on its general magnitude and on its precise value, harmonics at higher frequency numbers giving better mixing than non-harmonic frequencies. The range and accuracy of the frequency numbers in this study was limited by the pumps' characteristics. As expected however, the best mixing was achieved when the pumps pulsed synchronously.
- 3) The phase angle had a greater effect at the harmonic values of the frequency number with this effect diminishing as the pulse number increased. For non-harmonic frequencies this independence of the phase angle is explicable since, for example with the frequency number set at 0.75, although the pumps may begin a cycle in phase, the second and third injections will be  $120^\circ$  and  $240^\circ$  out of phase and the effect on the degree of segregation of a small change in one of these angles will be compensated for by the corresponding change in the other two.



- 4) A high systolic-diastolic duration ratio should produce a large axial spread of the injection pulse, however in this study no effect of the main fluid pulse shape on the degree of segregation was observed.
- 5) The Reynolds number, although important in steady flow, has little influence in pulsatile flow, with pulsatile flow being capable of producing a higher quality of mixing at low Reynolds numbers.

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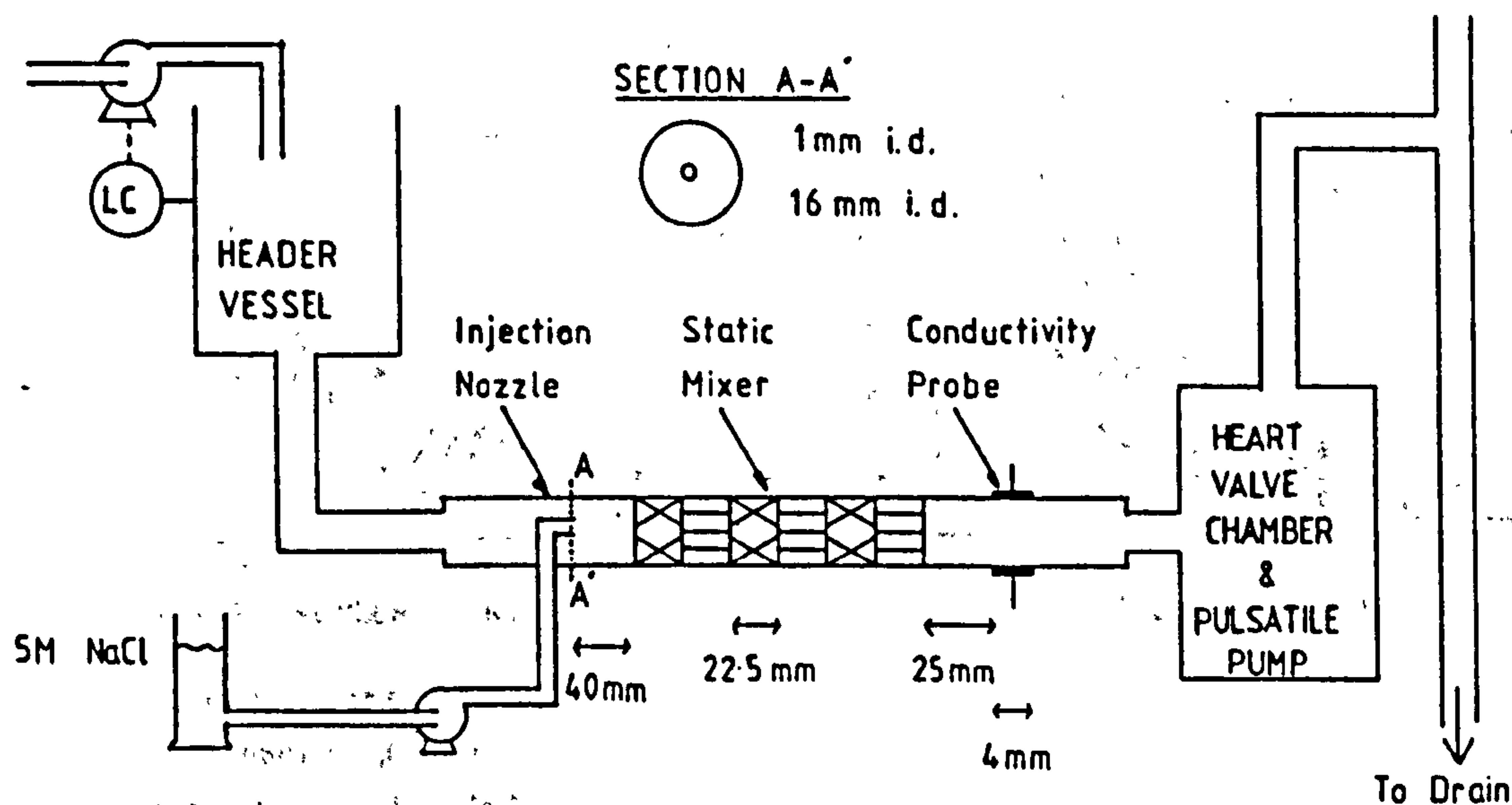
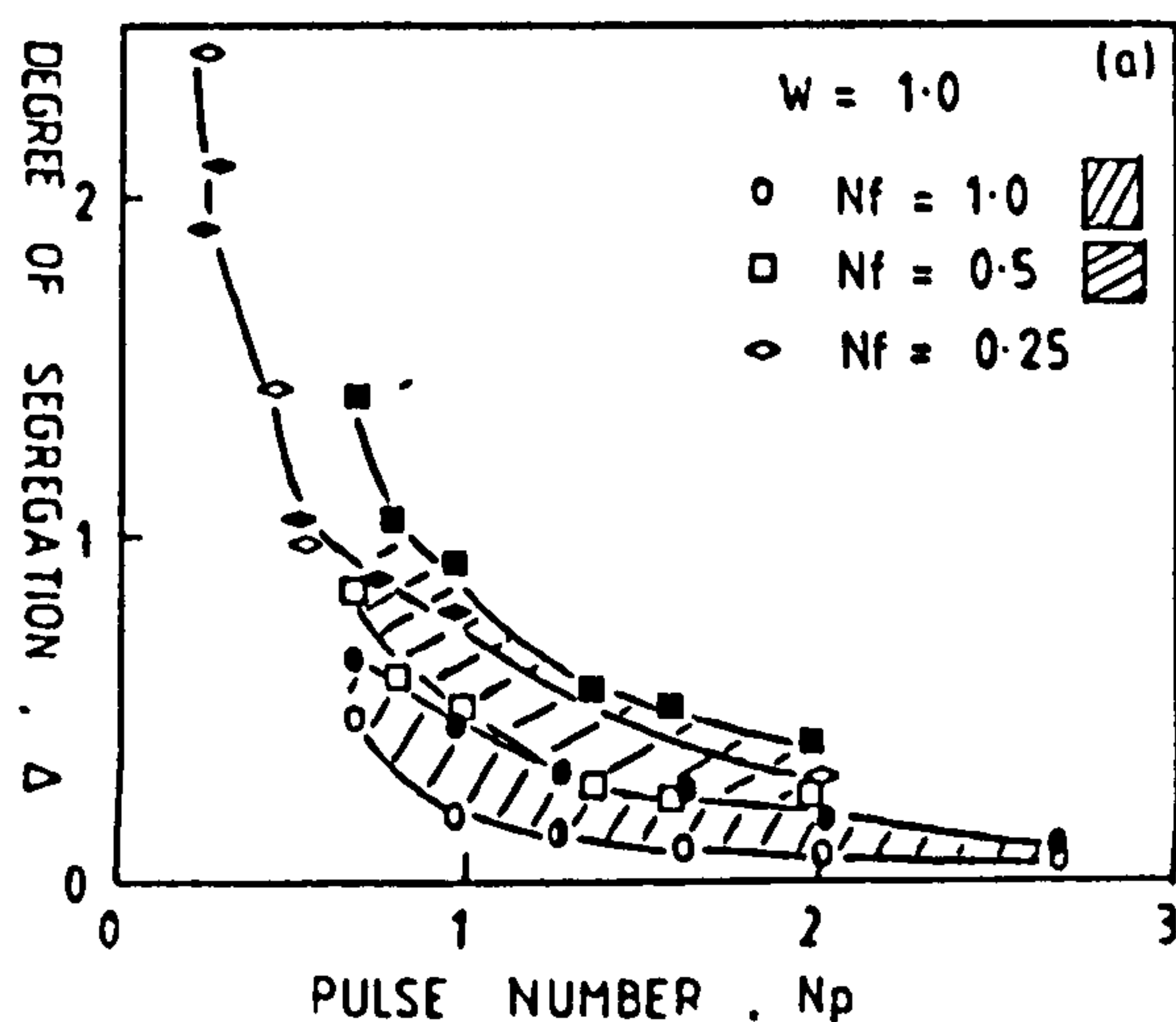
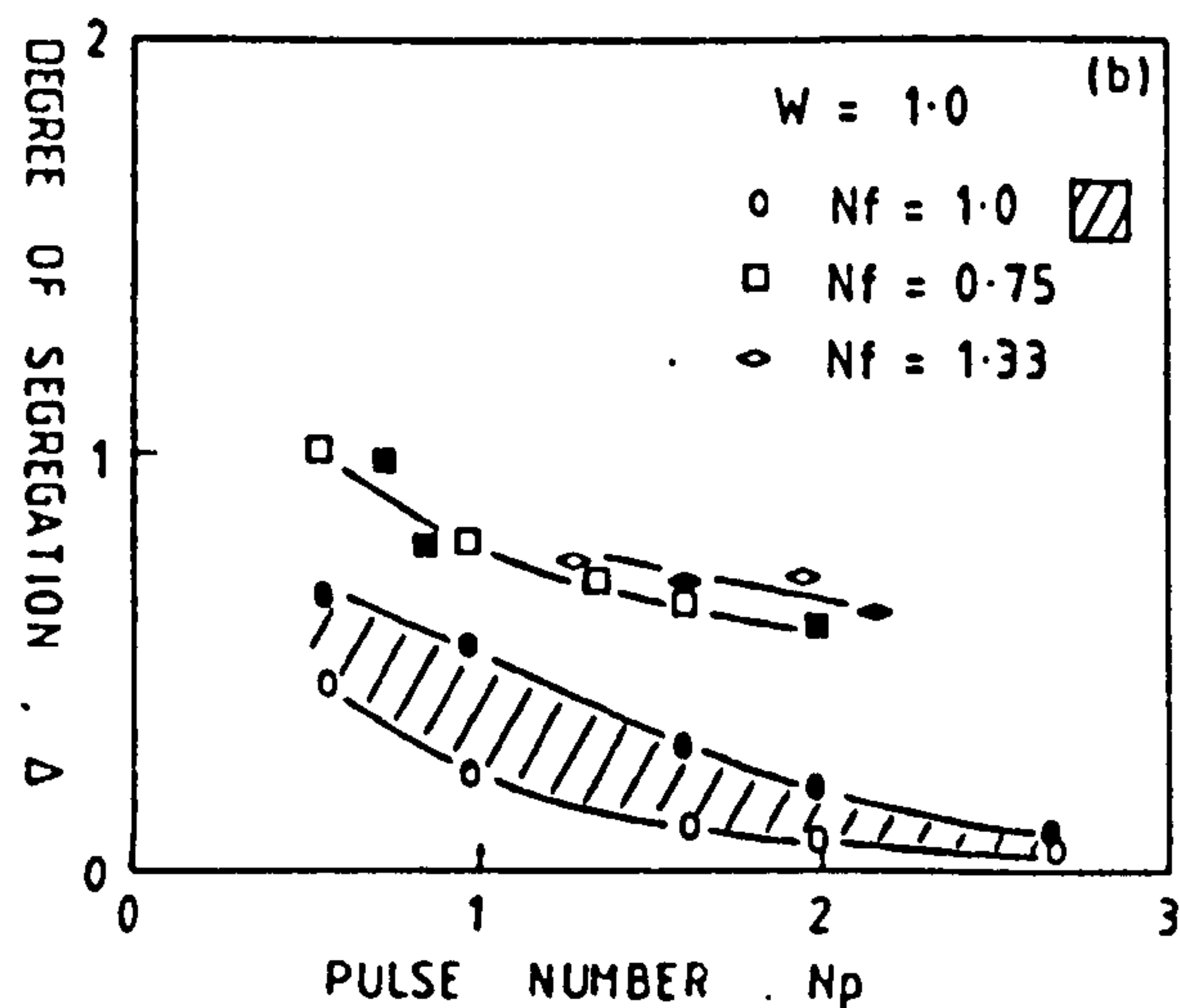


Figure 1

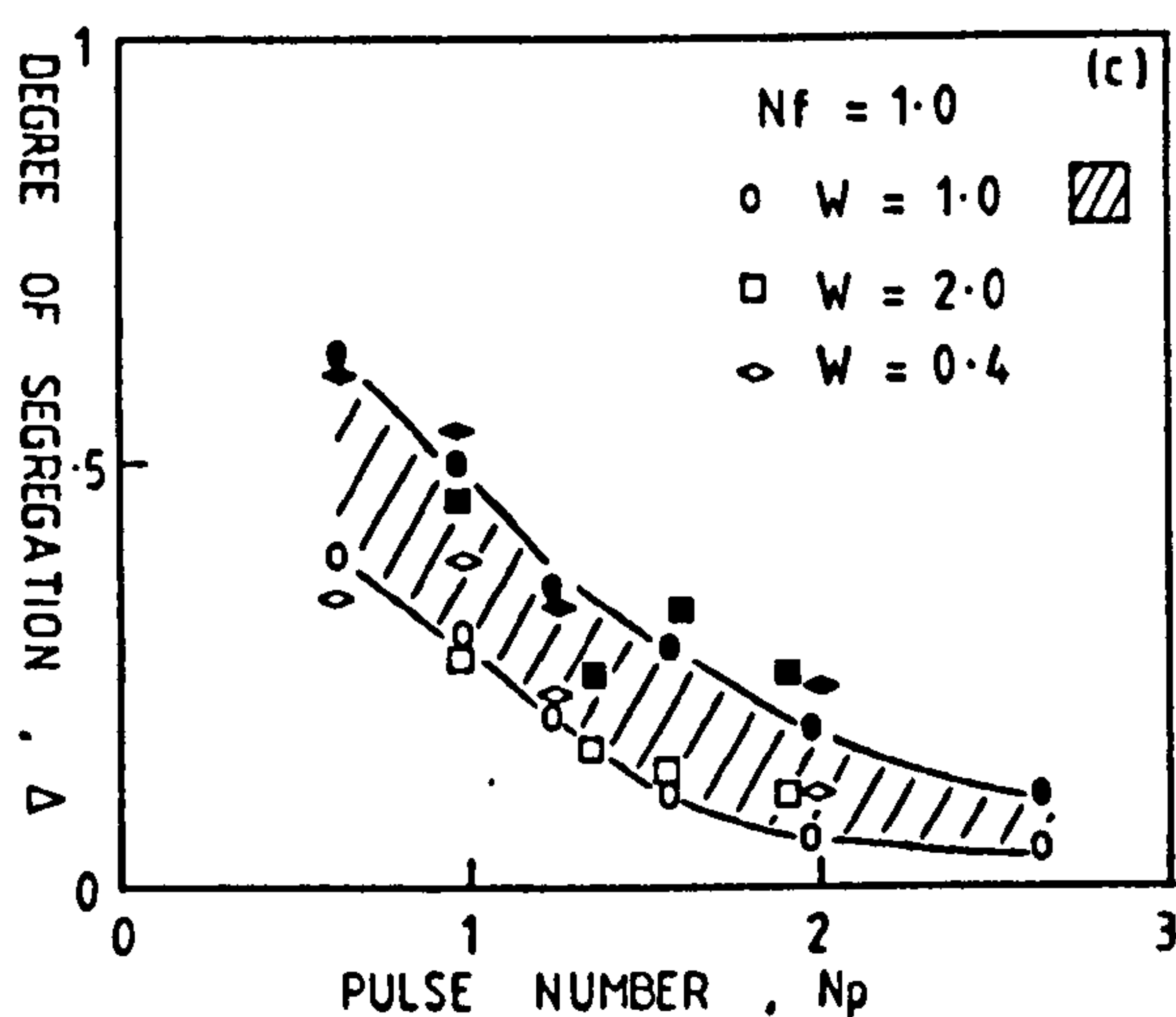
Schematic Diagram of Test Section



a) Effect of Harmonic Frequencies

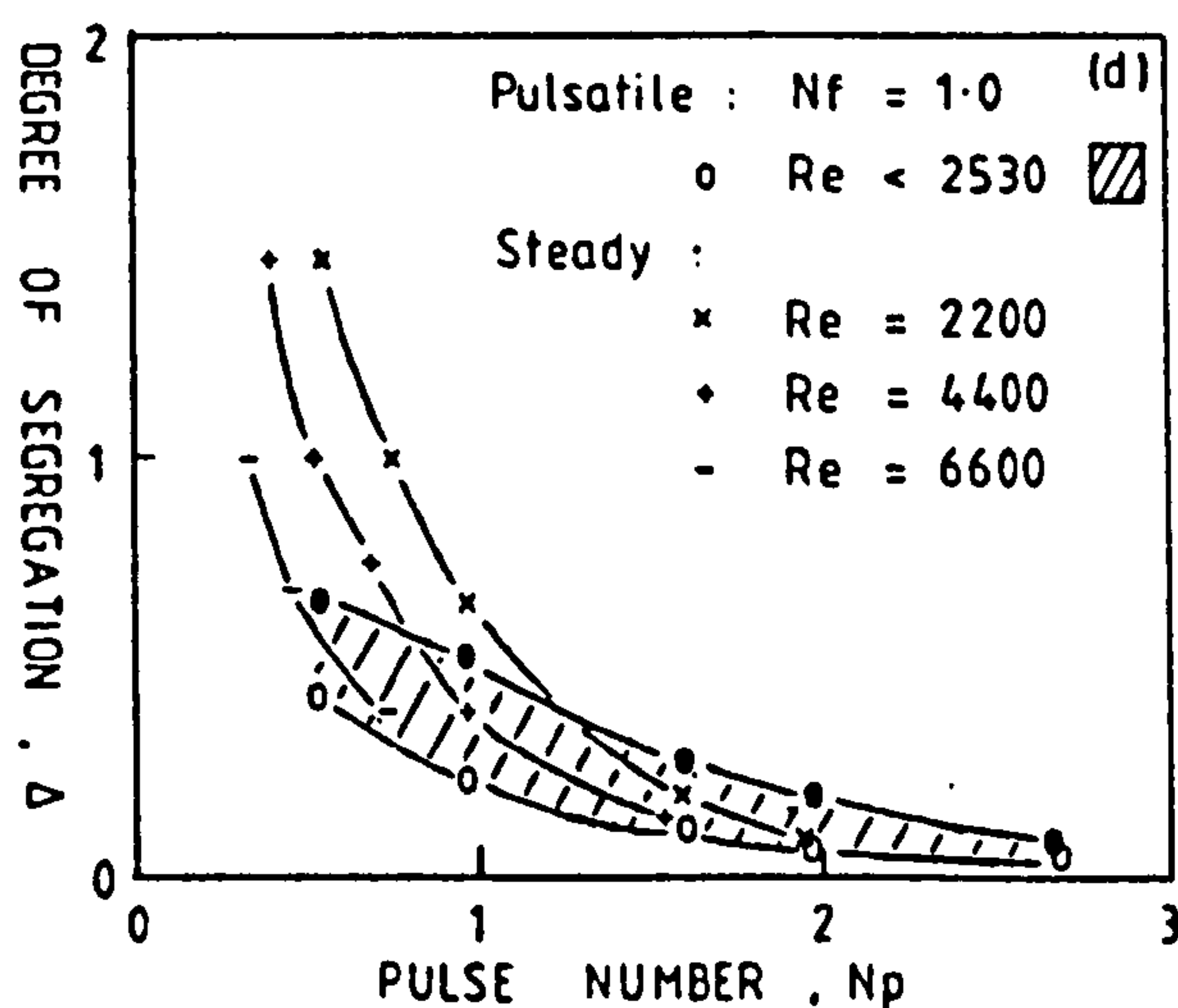


b) Effect of Non-Harmonic Frequencies



\* The scale varies for  $\Delta$ .  
 Open shapes:  $\phi = 0^\circ$   
 Closed shapes:  $\phi = 180^\circ$

c) Effect of Main Pulse Shape



d) Steady Versus Pulsatile Flow

Figure 2

Degree of Segregation Against Pulse Number for Various Operating Conditions



## **Appendix C**

### **Operating Instructions for the Milk Rig**

## OPERATING INSTRUCTIONS FOR RENNETISED MILK RIG

- 1) Place 3, 5 Gallon containers of milk on the trolley and, ensuring that all the valves are closed, connect the outlets from the containers into the main flow line.
  - 2) Ensure that the valve to the water container is open and that the container is full.
  - 3) Open the valve on the water line to the ejector.
  - 4) Open the mains water supply valve to the water container - adjusting it to give a slight overflow (it is prudent to flush some water down the sink to ensure that no sediment enters the water tank).
  - 5) When water starts to appear in the perspex vacuum chamber start up the 'Milk Pump'. The level controller should be set to read 40%.
  - 6) Once the level in the header vessel has risen to over 50% start up the pulsatile pump. The frequency knob should be adjusted to 72 to give 70 strokes/min and the stroke volume screw adjusted to 1.3 giving an overall flowrate of 2l/min.
  - 7) Switch on the 'water pump' ensuring that all the heater switches are 'on'. When the water inlet temperature to the heat exchange reaches  $30^{\circ}\text{C}$  turn off those heaters that are not required (for 2l/min only, the top switch should be 'on').
  - 8) Once the milk temperature has stabilised at the set value of  $30^{\circ}\text{C}$  and thermocouple number 5 is registering a constant value, check that no air is trapped in the milk line. Use wooden blocks if necessary to percussively dislodge any air bubbles adhering to the perspex. Open the water tap leading to the drain-pipe.
  - 9) The apparatus is now fully operational.
- Running
- 10) Close the mains water supply valve and the outlet valve from the water container. Open the valve on the milk container nearest to the heat exchanger.
  - 11) Allow milk to reach the test section and to run through it for approximately one minute before starting the dosing pumps. Record the time.
  - 12) When the level of milk in the first container has dropped to the level of the outlet close the outlet valve, and open the valve on the next container to the left. Use the funnel to pour the



remaining milk from the previous container into the one now in use. Flush the empty container out with hot water (To disconnect the container close both valves at its outlet and disconnect the line between the two valves - (Reverse of procedure 1)).

13) Repeat (12) for the second change-over.

#### Shut Down

14) When the third (or last) container is nearly empty use the wooden blocks to tilt it forward so as to extract as much milk as possible.

15) When it is empty close its outlet valve and re-open the valve from the water tank. Open the mains valve to this tank.

16) Once water appears in the line to the header vessel switch off the closing pumps. Record the time. Also switch off the 'Milk Pump' and 'Water Pump'. Close the water valve to the ejector and the mains valve to the water tank.

17) After a minute, or when the header tank level has dropped below 10% stop the pulsatile pump.

18) Dismantling of the test equipment should be carried out very carefully so as not to dislodge any clot. Remove the tube leading to the drain-pipe in the sink from the top of the test section. Switch off the water tap leading to this waste pipe.

19) Loosen the bolts securing the top 6" section to the rest of the test section and allow the milk to drain slowly from the flange. Use the cloths provided to soak up this milk. Remove this section carefully.

20) Repeat (19) for the second 6" section. The valve can now be observed in situ. Photographs should be taken before it is removed from the section (unless this can be done without damage to the clot).

21) Once the valve has been photographed, weigh the valve with the clot attached.

22) Clean as much of the test section as possible with water and then reassemble it. Follow steps 2-6 to flush out the system with water. Switch on the 'Water Pump' with all of the heater on and set the temperature control to 55°C.

23) Once thermocouple 5 is reading 55°C, stop the water flow, ensuring that the 'Water Pump' and the ejector are also switched off.

24) Disconnect the tube from the drain-pipe and insert it into the header vessel. Add 35g of Tergazyme to the water in the header vessel and start up the pulsatile pump. Leave running for 1 hour.

25) At the end of the day the Heating and Deaeration section of the rig should also be cleaned. Drain water from the storage vessel until the depth in that vessel is 6" connect the inlet tube to the header vessel to the spare length of silicon rubber tubing and insert the other end of this tubing into the water container. Start up the 'Milk Pump' and the 'Water Pump' (the level controller may have to be reset so as to give a steady flow of water). The valve to the ejector should also be opened. When the water has reached 50°C add 50g caustic soda (ODC) to the water. Leave circulating for one hour. Drain off some of this caustic solution to clean the milk containers.

26) Once the cleaning cycle is complete flush out the system with water for approximately half an hour. This may be done with the water temperature set at 30°C in preparation for another run.



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